JOURNAL OF AGRICULTURAL RESEARCH

VOL. 64 WASHINGTON, D. C., MARCH 15, 1942 NO. 6

CHANGES INDUCED IN THE BLOOD CELLS OF THE SOUTHERN ARMYWORM (PRODENIA ERIDANIA) BY THE ADMINISTRATION OF POISONS [1]

BY J. FRANKLIN YEAGER, *senior entomologist*, and SAM C. MUNSON, *junior zoologist, Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

The results of a study of a histochemically detectable polysaccharide, considered to be glycogen, in the blood cells of unpoisoned southern armyworms (*Prodenia eridania* (Cram.) have been presented in a previous paper (*31*).[2] The present paper describes the effects upon the blood cells and blood-cell glycogen of administering poisons to the larvae of that insect. The poisons used were nicotine bentonite, nicotine peat, rotenone, pyrethrum, phenothiazine, barium fluosilicate, sodium fluoaluminate, sodium fluoride, mercuric chloride, calcium arsenate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate. This investigation was qualitative and the results are reported as first approximations only.

MATERIALS

The nicotine bentonite and the nicotine peat each contained about 10 percent of nicotine. The rotenone was of a c.p. grade. The pyrethrum consisted of powdered pyrethrum flowers containing about 0.66 percent of pyrethrins I and II, including approximately 0.29 percent of pyrethrin I. The phenothiazine was approximately 99.5 percent pure. The barium fluosilicate was a fine-grade commercial product. The sodium fluoaluminate was synthetic and contained some uncombined aluminum oxide. The sodium fluoride conformed to the specifications of the American Chemical Society. The mercuric chloride was of a U. S. P. grade. The calcium arsenate was of average commercial grade, containing about 70 percent of arsenate and about 4 percent of arsenite. The calcium arsenite was about 85 percent pure. The arsenic trioxide was of a relatively pure grade. The paris green was a fine fraction separated from a commercial lot. The lead arsenate was a high-grade product. Administration of these poisons was by feeding. Nicotine was also administered as a vapor.

The insects used were sixth instars of the southern armyworm, reared in cages kept at approximately 25°–30° C. in winter and slightly higher in summer. The larvae were apparently in healthy condition.

[1] Received for publication July 31, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 331.

METHODS

SAMPLING, SMEARING, FIXING, AND STAINING OF BLOOD

Sampling, smearing, fixing, and staining of blood were performed in the manner already described (*31*). To prevent the blood cells from undergoing form changes during sampling and smearing, larvae were heat-fixed by immersion in water at 60° C. for 5 minutes. The heat-fixed blood, obtained by piercing a proleg, was smeared on cover slips, air-dried, and stained by the Bauer technique, with thionin as a counterstain. The glycogen method of Best, the mucicarmine method of Mayer, and the method of salivary counterproof were not used in this investigation, but a number of duplicate blood preparations were stained for glycogen by the Lugol iodine method. The blood smears were made from larvae that remained alive until heat-fixed, whether or not they had received poison. Larvae that showed no body movement, no regurgitation, and no defecation upon being heat-fixed were considered to be dead and were not utilized.

NONLIGATURED LARVAE

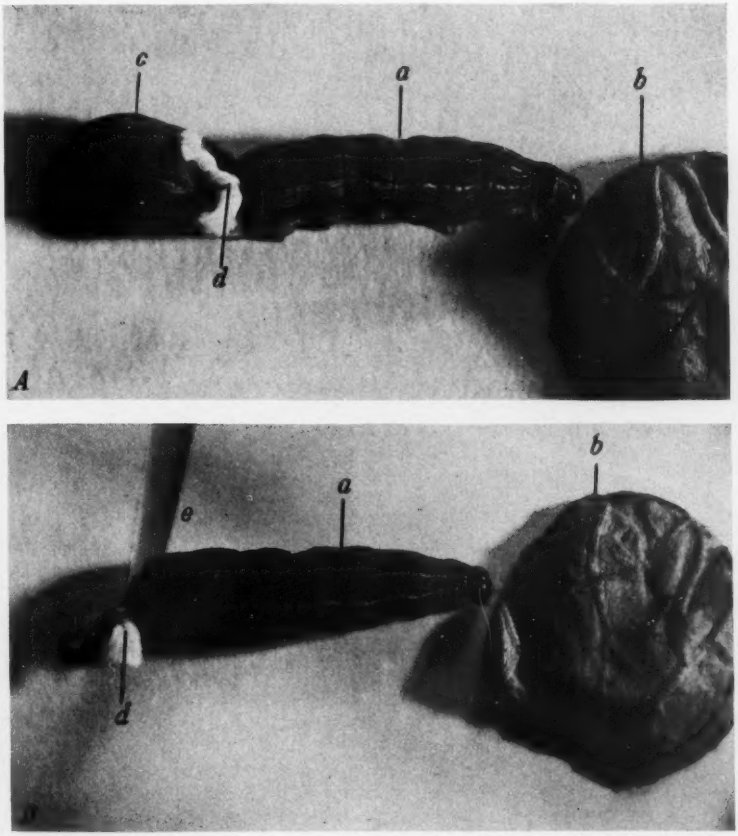
In a few experiments larvae were fed turnip-leaf sandwiches containing cornstarch paste (previously boiled) and glucose, to build up blood-cell glycogen (*31*). Some of these larvae, used as controls, were fed turnip-leaf-cornstarch sandwiches without the glucose. Others were fed turnip-leaf-cornstarch sandwiches without the glucose but with a large amount of a poison mixed with the cornstarch paste. After the larvae had been allowed access to the unpoisoned and poisoned foods for various periods ranging approximately from 1 to 30 hours, blood smears were made and stained for glycogen by the Bauer method.

In other experiments larvae were subjected to nicotine vapor at room temperature by being placed upon a wire-mesh platform within a closed Petri dish, the bottom of which was covered with liquid nicotine. They did not touch the liquid. The air in the Petri dish was considered to be approximately saturated with the nicotine vapor. Control larvae were similarly placed in Petri dishes but without nicotine. The larvae subjected to nicotine behaved abnormally and, after an exposure of 23.5 or more hours, appeared shrunken and somewhat dehydrated. The controls, however, continued to appear normal. Several other larvae, placed for 24 hours in a desiccator over calcium chloride, appeared dehydrated but not so abnormal as those subjected for 23.5 hours to nicotine vapor. Blood smears from all these larvae were stained for glycogen.

LIGATURED LARVAE

In experiments involving ligatured larvae, the insects were first given access, usually for 16.5 hours (overnight), to turnip-leaf-cornstarch-glucose sandwiches to build up blood-cell glycogen, and immediately thereafter they were starved for about 2 hours. The larvae were then ligatured, and some were used as ligatured controls and others as ligatured poisoned larvae. The latter will be referred to as poisoned larvae whether or not poison administration was followed by hematological changes.

The ligature consisted of a string tied tightly about the larva's body, separating it approximately into an anterior (fore) two-thirds



Ligatured larvae of *Prodenia eridania*. The fore end of each ligatured body is shown at *a*, near a turnip-leaf sandwich, *b*; the ligature is at *d*. In *A* the hind end is at *c*; in *B* it is on the other side of the cardboard stock, *e*, which encircles the body at the ligature.

to three-fourths and a posterior (hind) one-third to one-fourth portion. Ligaturing caused regurgitation, defecation, and loss of coordination between the anterior and posterior parts of the body. Two ligatured larvae are shown in plate 1.

The ligatured control larvae were given access to unpoisoned turnip-leaf-cornstarch sandwiches for periods ranging from 4.5 to 28 hours, after which they were heat-fixed. The ligatured larvae to be poisoned were given access to turnip-leaf sandwiches containing cornstarch and a large quantity of poison for periods usually ranging from 3 to 28 hours, although the time was longer for several larvae. Blood smears from the fore and hind parts of the ligatured bodies of both the control and the poisoned larvae were then stained for glycogen.

The ligatured larvae ate unpoisoned food moderately well, but it was more difficult to get them to eat sandwiches containing lead arsenate, sodium fluoride, arsenic trioxide, mercuric chloride, and pyrethrum. When the sandwiches contained nicotine peat, nicotine bentonite, or, particularly, rotenone, the larvae seemed to eat about as readily as the control larvae ate unpoisoned sandwiches. The sandwiches that contained the remaining poisons were eaten less readily.

Because of the tendency of the larvae not to eat mercuric chloride and pyrethrum, these poisons, moistened with water, were applied with a brush to the mouth parts and the anterior legs of some of the larvae, which were thus made to ingest some of the material. Some of the larvae given these two poisons were held in cardboard stocks (pl. 1, *B*), which acted as shields preventing the poison from coming in contact with the hind parts of the ligatured bodies.

GLYCOGEN COUNTS AND INDICES

Glycogen counts and glycogen indices were determined as described in a previous paper (*31*). A glycogen index of 0 means that less than 1 out of 400 blood cells contained glycogen inclusions that were visible with the optical system used.

This investigation was begun without knowledge as to whether adequate glycogen counts and glycogen indices could be obtained. The experiments were performed with no particular intent of subjecting the results to statistical analysis. Nevertheless, the groups of glycogen indices from the ligatured larvae were eventually treated as follows: The mean glycogen index of the hind ends (M_H), the mean of the fore ends (M_F), the mean difference between these indices (M_{H-F}), and the standard deviation of these differences (SD_{H-F}) were calculated for the group of ligatured control larvae and for each group of ligatured poisoned larvae. The differences (D) in the value M_{H-F} between each group of ligatured poisoned larvae and the group of ligatured control larvae were obtained. The standard errors of these differences (SE_D) were calculated. The statistical evidence of the effects or lack of effects of the poisons on blood-cell glycogen thus obtained is regarded as only supplemental to the results of the cytological observations.

RESULTS

The results of the experiments consist of observations of hematological and cytological changes that followed poison administration,

and of changes in glycogen counts and glycogen indices. The descriptions of microscopic changes represent what is considered to be a logical arrangement of the observed hematological facts.

GENERAL HEMATOLOGICAL CHANGES

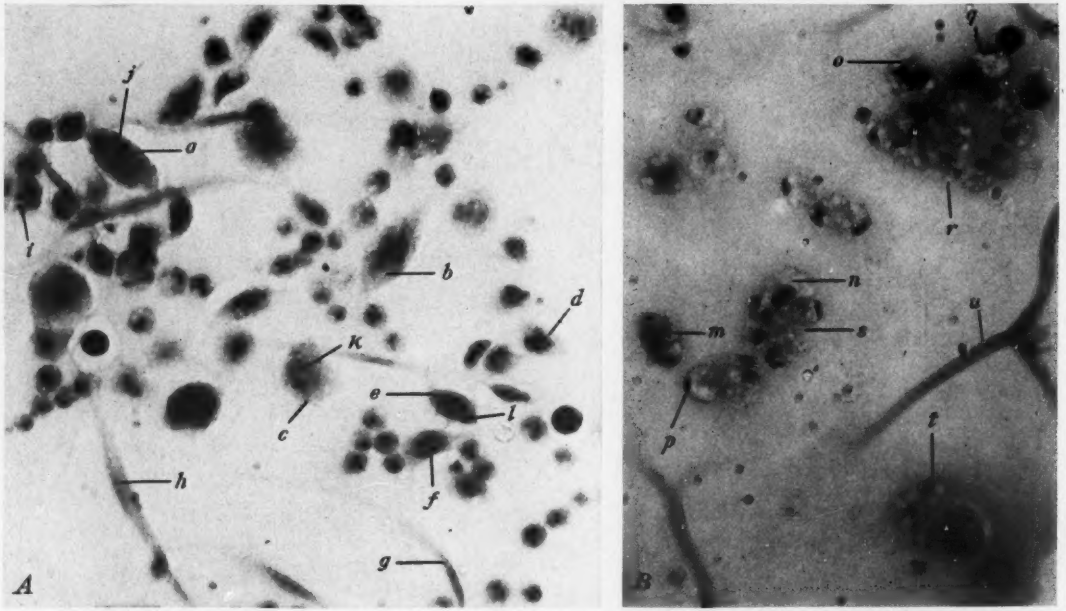
The blood cells of larvae treated with nicotine bentonite, nicotine peat, rotenone, phenothiazine, and pyrethrum appeared to be unaffected by these poisons, as indicated by comparison of the blood smears from the fore ends with those from the hind ends of the ligatured poisoned larvae and with those from the ligatured controls and the unligatured controls, although some blood cells showed a slight tendency toward passive-active changes, particularly when pyrethrum was used. The blood cells of larvae kept in concentrated nicotine vapor for 6 hours or less showed no effect of this treatment (pl. 2, *A*), but after 23.5 hours or more the blood cells of some of the larvae appeared abnormal relative to their controls, particularly with respect to rounding, agglutination, and increased vacuolization (pl. 2, *B*).

Administration of calcium arsenate, calcium arsenite, arsenic trioxide, paris green, lead arsenate, barium fluosilicate, sodium fluoaluminate, sodium fluoride, and mercuric chloride caused changes in the blood cells of the larvae receiving these poisons, which will be referred to in this paper as the effective poisons. Because individual larvae ingested different amounts of poisoned food, even when given access to it for the same period, no particular relationship between feeding period and hematological change was apparent. No attempt was made to determine the quantity of poison ingested or the relation between quantity ingested and hematological effect. The general hematological changes subsequent to poison administration, however, were recognized as progressive stages, which were designated as slight (+), moderate (++), and marked (+++). Plate 3, *A*, *B*, and *C*, and some of the other illustrations in this and in a previous paper (*31*) represent the normal (0) stage.

The slight, or +, stage of hematological change was characterized by a rounding up of most of the plasmatocytes and cystocytes, some cell agglutination, and perhaps an occasional spread form (pl. 4, *B*). The moderate, or ++, stage was characterized by the rounding up of the plasmatocytes and cystocytes, a considerable amount of cell agglutination, some cell distortion, and usually some cell disintegration (pl. 3, *D*). The marked, or +++, stage was characterized by the plasmatocytes and cystocytes having rounded or more or less spread forms, much cell agglutination, considerable cell distortion and disintegration, and frequently an obviously small number of cells in a smear (pl. 5, *B*, *C* and *E*).

Because the figures show only small areas of the blood smears from which they were taken, they do not identify the three stages so adequately as is possible by the more extensive microscopic examinations of the entire smears. Furthermore, the cytological details are not shown so clearly in the photomicrographs as they appeared in the smears. The figures, therefore, are presented only as illustrations, not as prototypes, of the three stages of general hematological change.

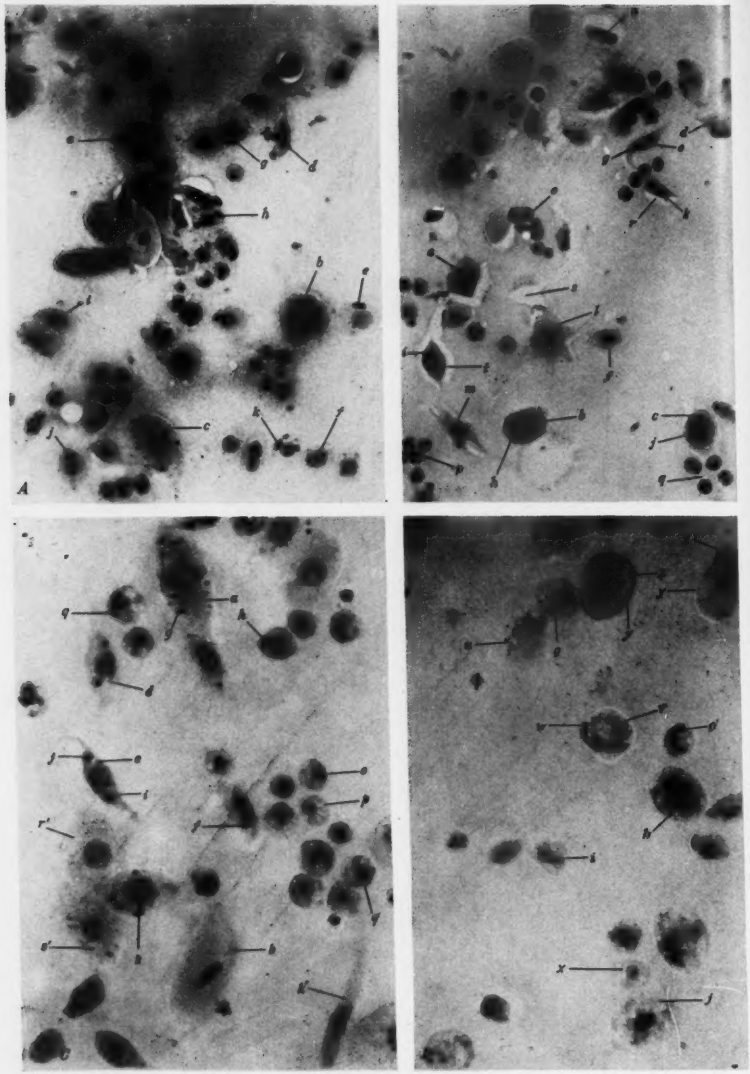
The terms employed in describing the three stages of general hematological change are used with the following meanings. Plasmatocytes and cystocytes are two types of southern armyworm blood cells that previously (*31*) have been identified among those that tend most



Parts of blood smears from unligatured larvae exposed to nicotine vapor: *A*, 6-hour exposure; *B*, 23.5-hour exposure. *a*, *b*, *c*, Cystocytes; *d*, *e*, *f*, plasmatocytes; *g*, *h*, nematocytes; *i–l*, glycogen inclusions; *m*, *n*, *o*, abnormal vacuolization; *p*, *q*, eccentric nuclei; *r*, *s*, *t*, abnormal agglutination; *u*, fold in plasma.

Glycogen indices: *A*, 38.00; *B*, 5.00 percent.

Bauer technique; 93× objective (oil); 10× ocular.

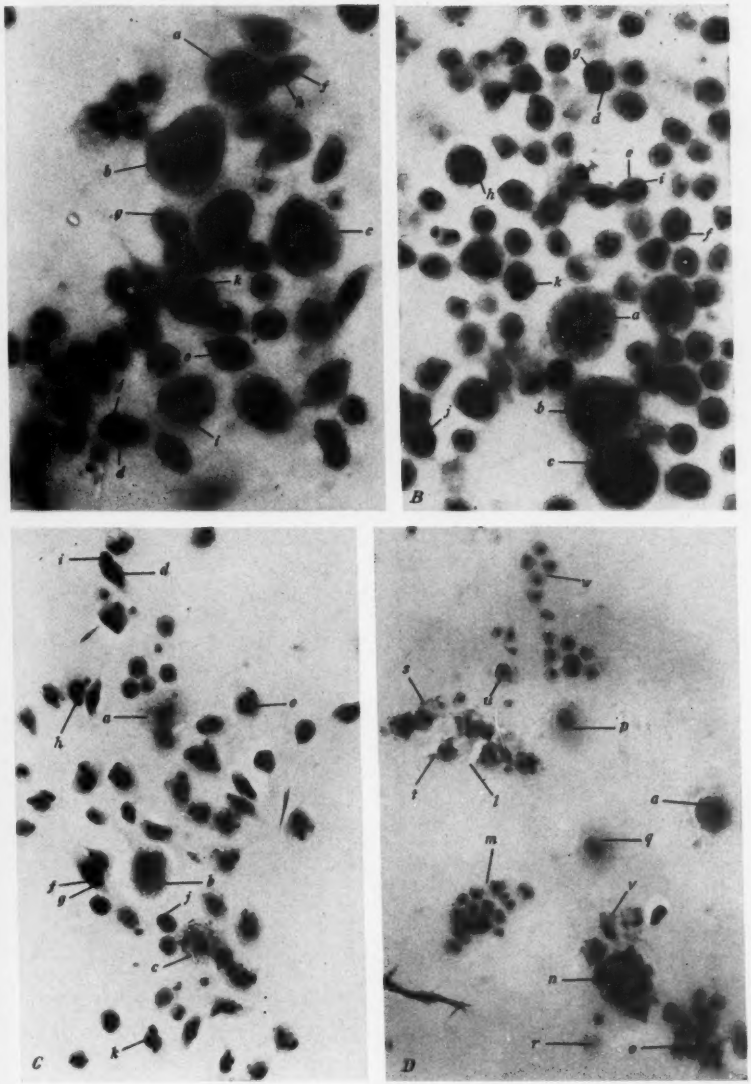


Parts of blood smears from a ligatured unpoisoned larva (*A*, hind end; *B*, fore end) and from a ligatured larva poisoned with mercuric chloride (*C*, hind end; *D*, fore end). *a*, *b*, *c*, Cystocytes; *d*, *e*, *f*, plasmatocytes; *g*–*k*, glycogen inclusions; *l*, *m*, polypodocytes; *n*, oenocytoid; *o*, *p*, *q*, spheroidocytes; *r*, *s*, *t*, breaks in thick smear of plasma; *u*, degenerating cell; *v*, rounded cell; *w*, eccentric nucleus; *x*, achromophilic cell; *y*, rounded, spread cells showing ectoplasm at *z*; *k'*, nematocytelike cell; *r'* and *s'*, cells with punctate nucleus.

Stage of general hematological change: *A*, *B*, and *C*, 0; *D*, ++.

Glycogen indices: *A*, 60.75; *B*, 86.75; *C*, 42.50; *D*, 18.25 percent.

Bauer technique; 93× objective (oil); ocular 10× (*A* and *B*), 15× (*C* and *D*).

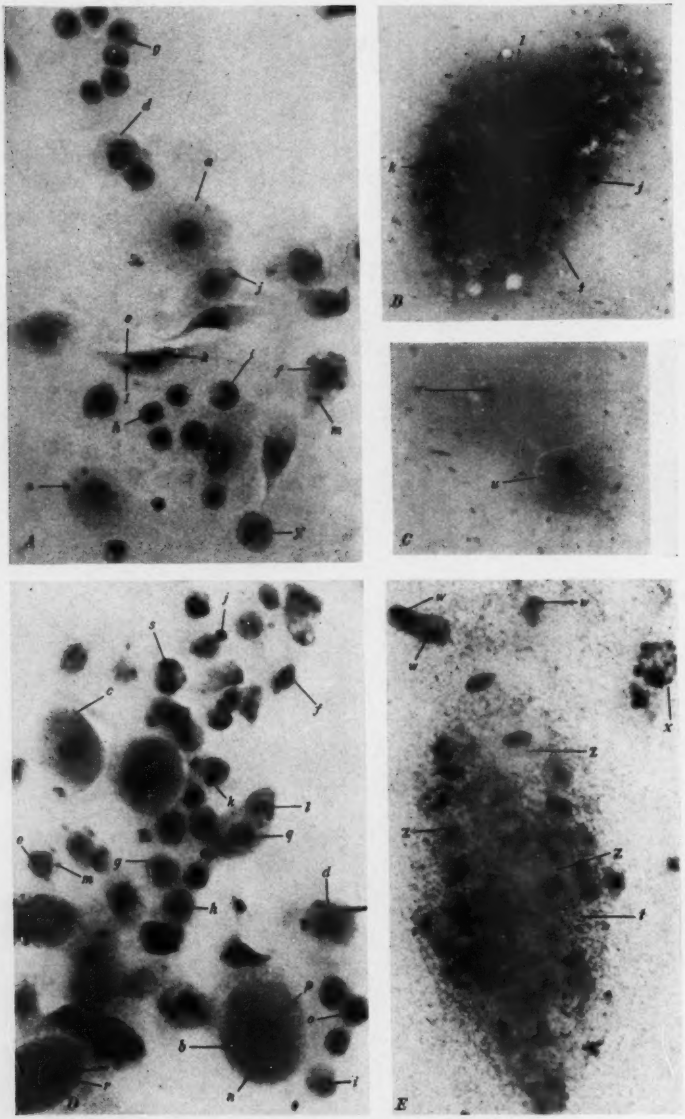


Parts of blood smears from two ligatured larvae poisoned with paris green: *A* and *B*, hind and fore ends of one larva; *C* and *D*, hind and fore ends of the other larva. *a*, *b*, *c*, Cystocytes; *d*, *e*, *f*, plasmatocytes; *g*–*k*, glycogen inclusions; *l*–*o*, abnormal agglutination; *p*–*r*, achromophilic cells; *s*–*v*, cellular deformation and plastid formation; *w*, undeformed but degenerating spheroidocytes.

Stage of general hematological change: *A* and *C*, 0; *B*, +; *D*, +++.

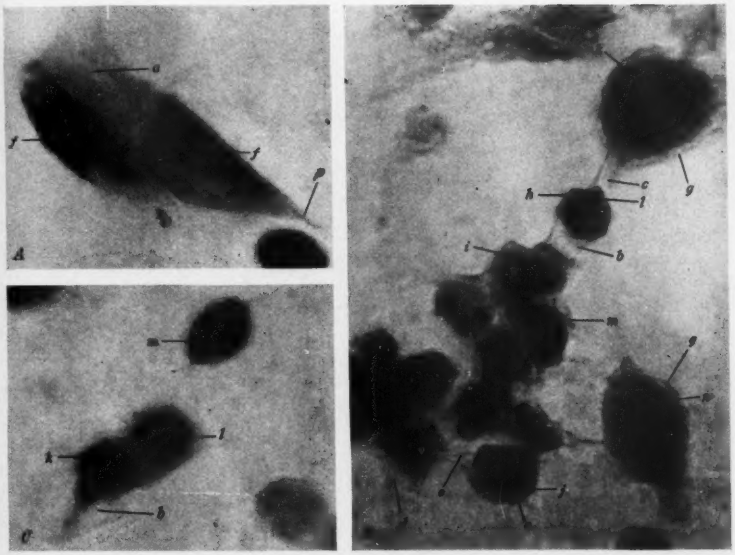
Glycogen indices: *A*, 38.75; *B*, 31.00; *C*, 67.25; *D*, 2.00 percent.

Bauer technique; 93× objective (oil); ocular 15× (*A* and *B*), 10× (*C* and *D*).



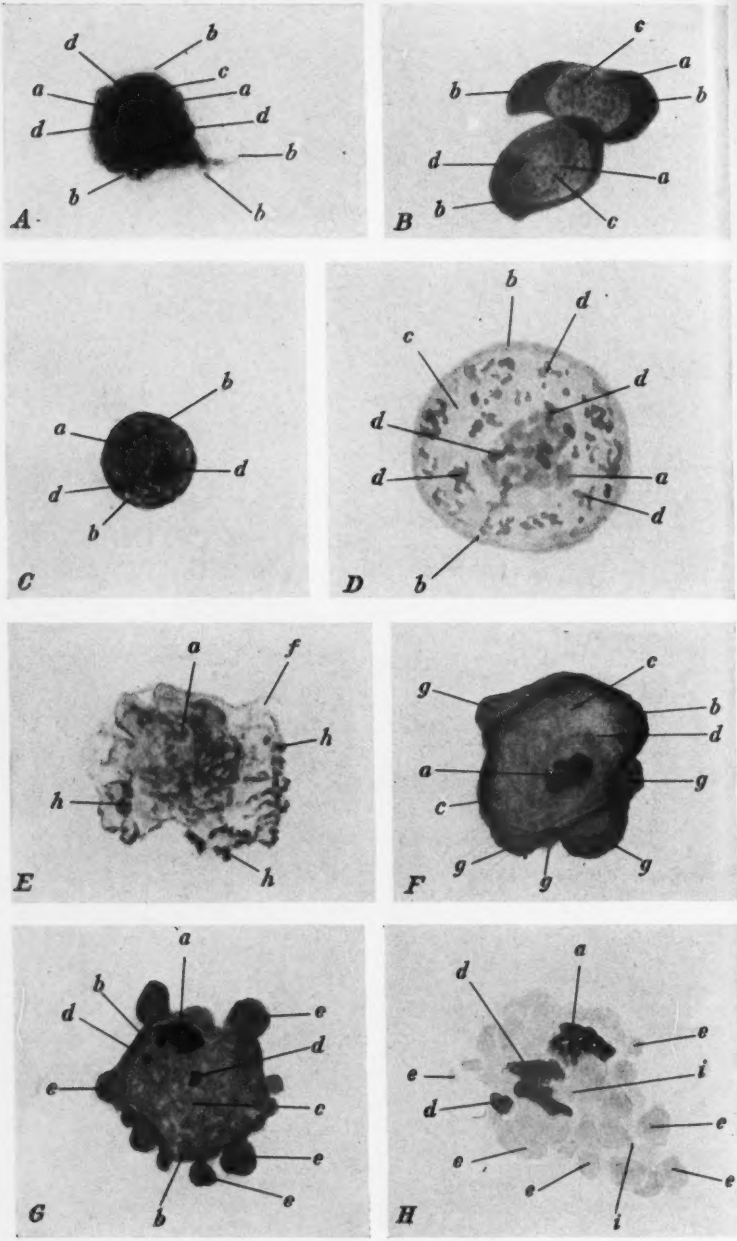
Parts of blood smears from two ligatured larvae poisoned with barium fluosilicate (*A*, hind end; *B* and *C*, fore end) and calcium arsenite (*D*, hind end; *E*, fore end). *a*, *b*, *c*, Cystocytes; *d*, *e*, *f*, plasmatocytes; *g*, *h*, *i*, spheroidocytes; *j*–*o*, glycogen inclusions; *p*, *q*, *r*, apparent prophases; *s*, anaphase; *t*, degenerating agglutinated cells; *u*, degenerating cystocyte; *v*, nearly completely degenerated cell; *w*, degenerating cells; *x*, degenerating cell showing plastid formation; *y*, metaphase; *z*, somewhat swollen translucent cells, probably cystocytes.

Stage of general hematological change: *A*, 0; *B*, *C*, and *E*, +++; *D*, 0 (?).
Glycogen indices: *A*, 34.00; *B* and *C*, 7.00; *D*, 43.75; *E*, 0.75 percent.
Bauer technique; 93× objective (oil); 15× ocular.



Parts of blood smears from ligatured larvae poisoned with mercuric chloride, showing cytoplasmic spreading: *A*, From the hind end, two plasmatocytes that appear to be in process of spreading (*a*) without losing their passive aspect or their general cytoplasmic structure and form; *B*, from the fore end, blood cells showing formation of thin ectoplasmic lamellae (*b–e*); *C*, from the fore end, three rounded plasmatocytes exhibiting ectoplasmatic activity (*b*). *f*, Nuclei; *g*, cystocytes; *h–j*, plasmatocytes; *k–o*, glycogen inclusions; *p*, spindle end of cell.

Stage of general hematological change: *B* and *C*, +.
Glycogen index: *C*, 28.25 percent.
Bauer technique; 93× objective (oil); 15× ocular.



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EXPLANATION FOR PLATE 7

Blood cells from the fore ends of ligatured larvae poisoned by barium fluosilicate (*A*, *B*), paris green (*C*, *D*, *F*, *G*, *H*), and mercuric chloride (*E*). *a*, Nucleus or part of nucleus; *b*, ectoplasm; *c*, endoplasm; *d*, glycogen inclusions; *e*, plastids; *f*, ragged ectoplasm; *g*, pseudopodialike bulges; *h*, granulation or minute plastids; *i*, remnant of main body of cytoplasm.

A, A rounded plasmatocyte showing irregularity of surface and extensions of ectoplasm, deeply stained endoplasm containing three glycogen inclusions, and the chromatin masses of the obscured nucleus.

B, Two plasmatocytes showing a pronounced demarcation between the homogeneous, relatively chromophilic ectoplasm and the endoplasm; widely spread chromatin masses of the nucleus are faintly visible in the endoplasm of each cell.

C, A rounded plasmatocyte, relatively chromophilic and containing two glycogen inclusions; cytoplasm partly obscuring nucleus and not particularly vacuolated but showing irregular intensity of stain; ectoplasm faintly distinguishable.

D, A cystocyte having a round, swollen, smooth-surface aspect, a partly obscured nucleus, many irregular glycogen inclusions, and an evenly distributed layer of ectoplasm.

E, A degenerating plasmatocyte showing cytoplasmic granulation (formation of minute plastids), cytoplasmic raggedness, and a somewhat distorted and relatively amorphous nucleus.

F, A cystocyte showing the formation of broad pseudopodialike bulges, a somewhat eccentric nucleus that shows a tendency toward pycnosis, and a faint remnant of a glycogen inclusion in the endoplasm, which is nearly structureless.

G, A rounded cystocyte showing plastid formation, a grossly punctate nucleus, and two remnants of glycogen inclusions in the endoplasm, which shows traces of cytoplasmic structures and a diffuse glycogen (Bauer) stain.

H, A degenerating plasmatocyte, with achromophilic cytoplasm, showing marked plastic formation, a somewhat ragged nucleus, and a remnant of the main body of the cytoplasm containing two glycogen masses.

Stage of general hematological change: *A*, *B*, and *C*, +; *D* and *H*, ++; *E*, *F*, and *G*, +++.

Glycogen indices: *A* and *B*, 25.50 (fore end) and 24.75 (hind end); *C*, 31.00 (fore end) and 38.75 (hind end); *D*, *F*, *G*, and *H*, 0 (fore end) and 21.75 (hind end); *E*, 12.75 (fore end) and 40.75 (hind end) percent.

Bauer-thionin technique; oil immersion; camera lucida wash drawings.

EXPLANATION FOR PLATE 8.

Blood cells from the fore ends of ligatured larvae poisoned with mercuric chloride (*A*, *C*, *D*, *F–J*) and paris green (*B*, *E*). *a*, Nucleus; *b*, plastid formation; *c*, gross vacuoles; *d*, glycogen inclusions; *e*, pseudopodialike bulges of cytoplasm; *f*, ectoplasm; *g*, endoplasm; *h*, nuclear extrusion; *i*, thionin-stained granules; *j*, vacuolization of glycogen inclusions; *k*, nuclear polysaccharide; *l*, a large central area (endoplasm?).

A, A large, degenerating cell of questionable identification showing marked plastid formation, faint indications of gross vacuolation of the cytoplasm and of some of the plastids, and a nucleus with an aspect suggestive of mitosis.

B, A degenerating, achromophilic plasmatocyte showing marked plastid formation.

C, A degenerating, rounded plasmatocyte with marked cytoplasmic vacuolization.

D, A degenerating, rounded plasmatocyte with an eccentrically located pycnotic nucleus, cytoplasmic pseudopodialike bulges, and glycogen inclusions.

E, A degenerating cystocyte showing partial extrusion of a somewhat pycnotic nucleus.

F, A large round, swollen cystocyte showing nuclear extrusion.

G, Either a large plastid or an enucleated plasmatocyte that contains a single glycogen inclusion.

H, A rounded plasmatocyte showing nuclear fragmentation.

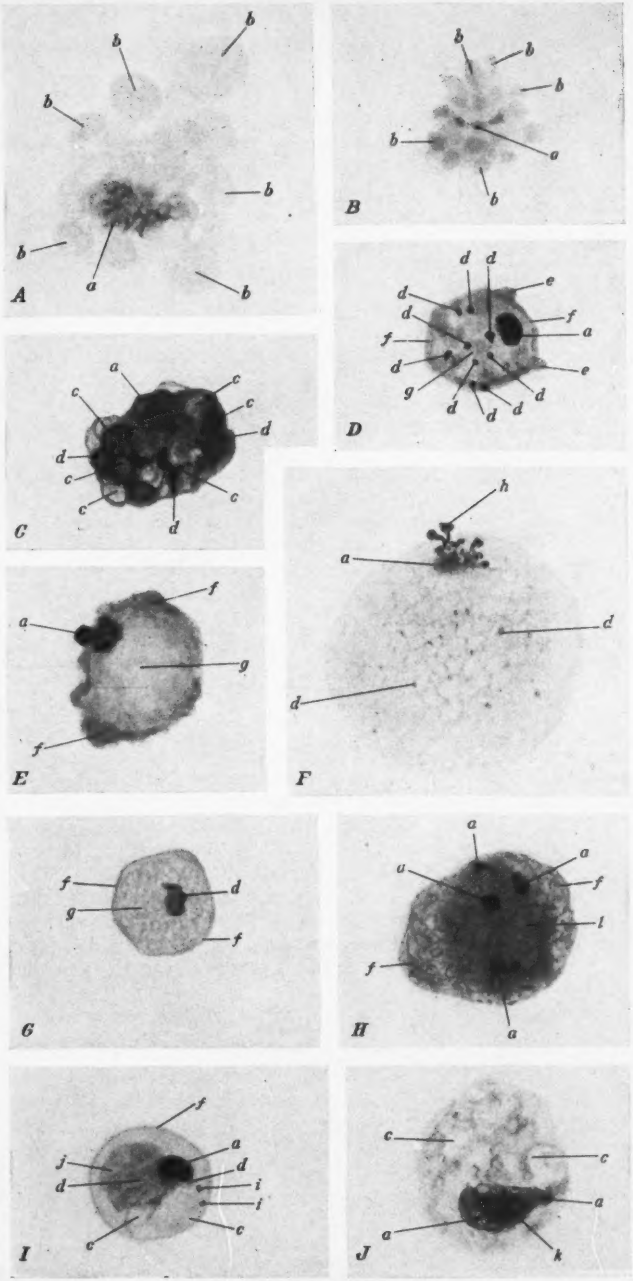
I, A round, swollen blood cell of problematical identification, although probably a plasmatocyte, containing glycogen inclusions.

J, A cell of problematical identification with nuclear polysaccharide, probably glycogen, cytoplasmic achromophilia, loss of structural organization, and gross, irregular vacuolization.

Stage of general hematological change: *A*, *B*, *C*, *E*, *F*, *H*, *I*, *J*, +++; *D*, *G*, ++.

Glycogen indices: *A*, *C*, *F*, *H*, *I*, *J*, 12.75 (fore end) and 40.75 (hind end); *B* and *E*, 0 (fore end) and 21.75 (hind end); *D* and *G*, 18.25 (fore end) and 42.50 (hind end) percent.

Bauer-thionin technique; oil immersion; camera lucida wash drawings.



(See explanation on opposite page)

readily to contain glycogen inclusions (pls. 2, *A;* 3, *A*, *B*, and *C;* 4, *A*, *B*, and C; 5, *A* and *D*; 6). Rounding up is illustrated by the withdrawal of the spindle ends of a fusiform cell, the contour of which usually tends to become but may not actually become circular (pl. 4, *A* and *B*). Cell agglutination, or coagulation, applies to the aggregation of certain cells into clumps, such as occurs during the clotting of insect blood (*30*, *32;* pls. 4, *D;* 5, *B* and *E;* 6). Distortion designates the occurrence of cellular forms that are apparently not characteristic of normal blood cells in either their passive or their active state (pls. 7, *F;* 8, *E*). Disintegration refers to the more advanced stages of degeneration, in which the cell may show a tendency to lose part of its protoplasm or may become more or less ragged, broken down, or structureless (pls. 4, *D;* 7, *E*, *G*, and *H;* 8, *A*, *B*, *I*, and *J*). Spread form is that form characteristically associated with the active state of certain blood cells, in which they tend to become thinner and to occupy greater areas of the surfaces on which they rest, and in which their homogeneous, peripheral cytoplasm, or ectoplasm, frequently becomes quite distinguishable from their heterogeneous endoplasm, which contains nucleus, vacuoles, glycogen inclusions, and other formed or included bodies (pls. 3, *D*, *y;* 6, *B* and *C;* 7, *B*).

Each of the smears that showed the effects of poison administration presented blood pictures that were in accord with the progressive stages of general hematological change, whether the smear was from a ligatured or a nonligatured larva. It should be emphasized, however, that the stages +, ++, and +++ have been described in very general terms. Apparently not all the blood cells of poisoned larvae, for example, underwent passive-active transformations in which they first rounded up and later spread.

CYTOLOGICAL CHANGES

The cytological changes that followed the administration of the effective poisons fall into three categories: (1) Passive-active transformation and cell agglutination, (2) possible regenerative changes, and (3) degenerative changes. Unless otherwise stated, this description is limited to the cystocytes and the plasmatocytes, because these cell types, particularly the cystocytes, could be readily identified in the Bauer-stained thionin-counterstained smears. As previously indicated (*31*), the cystocytes and the plasmatocytes are essentially fusiform cells, although some of them may assume roundish, ovoid, or polymorphic forms in the circulating blood.

Passive-Active Transformation and Cell Agglutination

Beginning with a spindle-shaped cystocyte or plasmatocyte, the changes described below were found to follow the administration of a sufficient quantity of an effective poison. The fusiform cells (passive form) tended to undergo transformation, which left them partly or completely in what were considered to be their active forms. During passive-active transformation a fusiform cell (pls. 3, *C*, *a*, *b*, and *e;* 4, *C;* 5, *A*, *e*) may withdraw its spindle ends and round up, assuming a more or less circular (pl. 4, *B*), ovoid (pl. 3, *D;* 7, *B*), or polymorphic (pls. 6, *B*, and *C;* 7, *A*) outline, or it may begin to spread without withdrawing its spindle ends (pl. 6, *A*). The transformation frequently involves augmented activity of the cytoplasm nearest the

cell surface, resulting in the development of surface irregularities and various extensions of the ectoplasm (pls. 6, *B* and *C;* 7, *A*).

After partial or complete transformation the cells presented variants of the following forms:

(1) Variably round or ovoid outline and exhibiting surface irregularities (pl. 7, *A*).

(2) More or less circular, smooth outline, without surface irregularities. The heterogeneous endoplasm could be readily distinguished from the surrounding homogeneous ectoplasm (pls. 3, *B*, *D*, *z;* 7, *B*), which varied from a very thin to a moderately thick layer, locally thinned or thickened into tonguelike or caplike enlargements, probably representing broad pseudopodial formations. In this form of cell the ectoplasm often tended to be more chromophilic than the endoplasm. Sometimes this cellular form presented a swollen or turgid aspect.

(3) Round or nearly round without marked distinction between endoplasm and ectoplasm and without particular surface irregularities (pls. 4, *B;* 7, *C*).

(4) More or less spread, having a round, ovoid, or irregular shape, and showing much evidence of surface activity, in the form of very thin, lamellar, ectoplasmic extensions or membranes of the same character as those shown in plate 6, *B* and *C*, some of which were relatively large.

(5) Tending to maintain a passive aspect, except that they appeared to have become much more spread upon the surfaces whereon they lay (pl. 6, *A*).

The cystocytes tended to assume forms (1), (2), and (3). The plasmatocytes tended to assume any one of the five forms.

Accompanying these changes and the subsequent degenerative changes, agglutination occurred involving various types of cells, among which were the cystocytes and the plasmatocytes (pls. 4, *D;* 5, *B* and *E;* 6, *B* and *C*).

Possible Regenerative Changes

Changes of a regenerative character may have occurred rather soon after poison administration. The possible regenerative changes consisted of an apparent increase in mitotic activity, made evident in the nuclei of some cells by their assuming an aspect suggestive or definitely indicative of the prophase of mitosis (pls. 5, *D*, *p*, *q*, and *r*). Other phases of mitosis were also observed in blood smears from both normal and poison-affected larvae (pl. 5, *D*, *s*).

Degenerative Changes

The cytoplasmic changes of a degenerative character include apparent swelling, disruption of and decrease in visibility of normal structure, achromophilia, decrease and loss of blood-cell glycogen, formation of broad pseudopodia or cytoplasmic bulges, plastid formation, excessive vacuolization, and raggedness. Nuclear degeneration involved distortion, raggedness, loss of normal structure, achromophilia, assumption of more or less peripheral positions, fragmentation, pycnosis, and extrusion.

The plasmatocytes and the cystocytes frequently exhibited what appeared to be a swollen aspect, which seemed particularly associated

with a rounded, smooth-surface, cellular form and occurred more conspicuously among the cystocytes (pls. 5, *E*, 7, *D;* 8, *F* and *I*). Whether an actual increase in cell volume or a relatively great spreading of the cell upon the supporting surface was involved was not determined. Various degrees of apparent swelling occurred.

Disruption of and decrease in visibility of cytoplasmic structures were observed among both cystocytes and plasmatocytes. In general, as the active degenerative changes progressed, the normal cytoplasmic structure tended either to become disrupted and to assume an abnormal appearance or to become less visible until it completely disappeared; sometimes both changes occurred (pls. 5, *E*, *e*, *f*, *g*, and *w;* 7, *D*, *F*, *G*, and *H;* 8). With complete disappearance of normal endoplasmic structure the cytoplasm tended to assume a more or less homogeneous aspect, in which case the peripheral ectoplasm might or might not be distinguishable from the endoplasm. Usually as degeneration proceeded many of the affected cells became relatively achromophilic.

During cytoplasmic degeneration a decrease of blood-cell glycogen occurred in many cells, and with sufficient cellular degeneration it eventually disappeared (pls. 7, *F* and *G;* 8). The disappearance of the glycogen inclusions sometimes seemed to involve an appearance in the endoplasm of a faint, diffuse positive reaction to the Bauer technique, indicating the possibility of glycogen occurring there in a diffuse state. In some of the cells some glycogen persisted into rather advanced stages of degeneration, as shown in plate 7, *H*.

Some degenerating cells underwent a sort of deformation in which broad bulges, suggestive of pseudopodia, occurred (pls. 7, *F;* 8, *D* and *E*). Sometimes only such broad cytoplasmic bulges occurred, but sometimes cells formed a greater number of smaller bulges or pseudopodia (pls. 4, *D*, *s*, *t*, and *v;* 5, *E*, *x;* 7, *G*), which gave the peripheries of the cells a very irregular aspect, as though the cells were extending tongues of cytoplasm from their surfaces. At more advanced stages of this process some of the protruded cytoplasmic tongues occasionally became nearly or quite detached from the rest of the cell and lay near the cell surface as free bodies, or plastids (pls. 7, *H;* 8, *A* and *B*). Sometimes in the final stages of plastid formation by a disintegrating cell, most of the cytoplasm appeared as a number of more or less separated plastids. Plastids frequently had a relatively homogeneous appearance.

Cells frequently took on a more ragged aspect as degeneration proceeded, the cytoplasm appearing irregularly frayed and torn.

Degenerating cells were sometimes grossly vacuolated to an abnormal degree (pl. 8, *C*).

Resting nuclei of the plasmatocytes and cystocytes normally possess a decidedly punctate structure (pls. 3, *C;* 5, *A* and *D;* 6, *A;* and some of the illustrations in *31*). During degeneration many nuclei tended to lose their punctate structure and became distorted, ragged, amorphous, and achromophilic (pls. 7, *D*, *E*, and *F;* 8, *A*, *B*, *F*, and *J*). Some nuclei became more or less pycnotic (pl. 7, *F* and *G*), variably assumed peripheral positions (pls. 7, *G;* 8, *D* and *I*), and in some cells became partially or completely extruded (pl. 8, *E*). Sometimes nonpycnotic nuclei tended to become extruded (pl. 8, *F*). Occasionally nuclei apparently being extruded seemed to be in a

mitotic condition (pl. 8, *F*). Large masses of cytoplasm, with or without glycogen inclusions but containing no visible nuclei, were occasionally observed. Some of these masses were too large to be considered as mere plastids and were thought to be cells that had lost their nuclei, either by extrusion or by degeneration. Plate 8, *G*, shows such a nonnucleated cell, or perhaps plastid, that contains a single glycogen inclusion.

Nuclei of degenerating cells sometimes appeared to break up into a number of discrete bodies. The fragmented nuclei showed varying tendencies to scatter (pls. 8, *H;* 9, *A*). Occasionally either whole nuclei (pl. 8, *I*) or nuclear fragments appeared as homogeneous droplets.

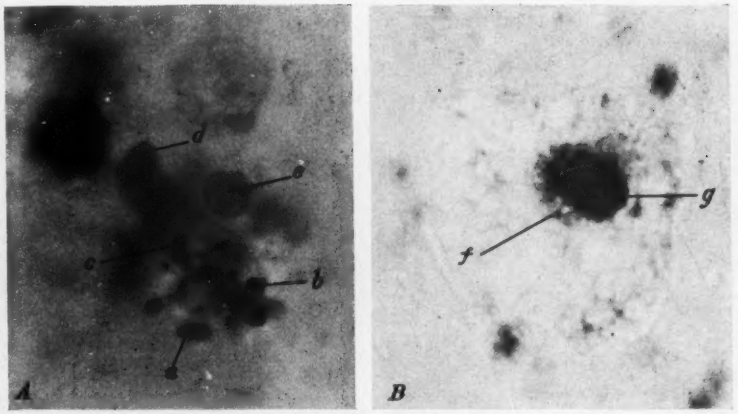
Other Observations

In some degenerating cells the glycogen inclusions looked as though they might have become aggregated or fused into somewhat larger masses. Glycogen inclusions sometimes appeared especially vacuolated (see also *31*), particularly when mercuric chloride was administered. Not all the cystocytes and plasmatocytes in a blood smear showed plastid formation, even when plastid formation was most pronounced. Although many of the pycnotic nuclei occupied eccentric positions in the cells, complete nuclear extrusion was observed infrequently. Blood smears from some of the poisoned larvae contained very large cells, identified as variably abnormal midgut epithelial cells that were often excessively vacuolated, bacteria that apparently had entered the blood stream from the ruptured gut (pls. 3, *D;* 5, *B*, *C*, and *E*), and other structures that were interpreted as partly digested fragments of leaf. Such tissue and intestinal debris were observed particularly in the blood of larvae poisoned with lead arsenate, arsenic trioxide, and the fluorides. In some of the blood smears from poisoned larvae, particularly from those given lead arsenate, dark thionin-stained spots or bodies occurred either in the plasma or associated with some of the cells. Infrequently a cell appeared to be in the process of extruding glycogen inclusions.

In some of the blood smears showing advanced cellular degeneration, from larvae poisoned with mercuric chloride, the nuclei of certain cystocytelike cells of questionable identification showed a positive polysaccharide reaction to the Bauer stain (pls. 8, *J;* 9, *B*). These cells seemed to be either modified cystocytes or transformed plasmatocytes. Because certain transitional forms seemed to link these questionable cells with the plasmatocytes, at least some of them were tentatively considered to be modified plasmatocytes.

Although plastid formation was observed to occur in larvae poisoned with the arsenicals and with mercuric chloride, it was not definitely observed in larvae poisoned with the fluorides, although some blood cells showed formation of broad pseudopodia. The most marked plastid formation was observed after administration of paris green. Abnormal vacuolization of blood cells was observed particularly after administration of mercuric chloride and the fluorides.

In contrast to the cystocytes and plasmatocytes, the spheroidocytes (pls. 3, *B*, *o*, *p*, and *q*; *C*, *o*, *p*, and *q*; 5, *A*, *g*, *h*, and *i*; *D*, *g*, *h*, and *i*; plates in *31*) underwent little or no change in form as degeneration proceeded. No glycogen was observed in any oenocytoid (pl. 3, *B*, *n:* plates in *31*), either normal or degenerate. Nuclear polysaccharide was detected only in the indeterminate cystocytelike cells that have



Blood cells from the fore ends of larvae poisoned with mercuric chloride: *A*, A cell showing nuclear fragmentation, as at *a*, *b*, and *c*; plastid formation is indicated at *d* and *e*. *B*, A cell showing in its nucleus (*f*) a positive reaction (*g*) to the Bauer stain for polysaccharide.

Both smears showed a +++ stage of general hematological change.
Bauer technique; 93× objective (oil); 15× ocular.

been described, and then only when mercuric chloride was administered.

CHANGES IN GLYCOGEN INDICES AND GLYCOGEN COUNTS

Glycogen Indices

The statistical values for the glycogen indices of ligatured control and ligatured poisoned larvae are given in table 1.

TABLE 1.—*Difference between the mean glycogen indices of the hind ends and those of the fore ends of ligatured larvae of Prodenia eridania after the administration of unpoisoned (controls) and poisoned food, and differences between the poisoned larvae and the controls*

Treatment	Larvae	Mean glycogen index		Difference between hind and fore ends		Difference between poisoned and unpoisoned larvae	
		Hind end (M_H)	Fore end (M_F)	Mean difference (M_{H-F})	Standard deviation (SD_{H-F})	Difference from control (D)	Standard error (SE_D)
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None (control)	21	32.33	35.58	−3.25	6.72		
Nicotine bentonite	11	53.18	54.66	−1.48	7.08	1.77	2.59
Nicotine peat	8	47.94	54.69	−6.75	17.34	3.50	6.30
Rotenone	8	18.69	24.19	−5.50	9.55	2.25	3.68
Pyrethrum	18	37.57	40.51	−2.94	11.27	.31	3.03
Phenothiazine	6	41.63	37.67	+3.96	6.05	7.21	2.87
Barium fluosilicate	13	32.90	24.32	+8.58	11.89	11.83	3.61
Sodium fluoaluminate	10	36.70	19.55	+17.15	15.22	20.40	5.03
Sodium fluoride	12	42.54	34.06	+8.48	10.18	11.73	3.28
Mercuric chloride	11	35.82	18.43	+17.39	20.96	20.64	6.49
Calcium arsenate	5	52.60	19.70	+32.90	5.07	36.15	2.69
Calcium arsenite	5	27.10	1.70	+25.40	14.23	28.65	6.53
Arsenic trioxide	15	49.62	34.95	+14.67	15.50	17.92	4.26
Paris green	9	37.66	17.91	+19.75	18.70	23.00	6.41
Lead arsenate	15	41.35	30.88	+10.47	16.76	13.72	4.57

It should be noted that the difference in the mean glycogen index between the hind ends and the fore ends (M_{H-F}) of the controls is a negative value, and that negative values of M_{H-F} were also obtained for nicotine bentonite, nicotine peat, rotenone, and pyrethrum, none of which, as has been stated, produced marked morphological changes in the blood cells. Although the M_{H-F} value for phenothiazine is positive, no definite hematological changes followed the administration of this poison. All the other poisons produced hematological changes, and all have positive M_{H-F} values.

Since the standard deviation of the hind-fore end differences (SD_{H-F}) of the controls is 6.72, the difference between the hind and the fore end ($H-F$) of a poisoned larva would have to be greater than +10.19 (i. e., 13.44−3.25) when 2 SD_{H-F} is taken or +16.91 (i. e., 20.16−3.25) when 3 SD_{H-F} is taken as a measure of significance, if the larva is to be regarded as significantly showing the result of poison administration by a relative decrease in fore-end glycogen index. The standard of 3 SD_{H-F} is used here. Only three poisoned larvae which had hind-end glycogen indices of 20 percent or more and whose hind-end and fore-end blood smears showed, respectively, 0 and ++ or 0 and +++ general hematological conditions showed differences between hind and fore ends of less than 17 percent. It was considered that in these experiments the $H-F$ difference in glycogen index for a poisoned larva must be at least +17 percent before the glycogen index of the fore end would show a significant relative decrease as a result

of poison administration. Inasmuch as this value is derived from the controls only, it should be considered as a minimum difference.

Three times SE_D is also taken as a standard of significance. Accordingly, administration of nicotine bentonite, nicotine peat, rotenone, pyrethrum, and phenothiazine is found to have no effect upon $H - F$ differences in glycogen index, since in each group D is less than 3 SE_D, whereas relative decreases in the fore-end glycogen index are found to follow administration of barium fluosilicate, sodium fluoaluminate, sodium fluoride, mercuric chloride, calcium aresnate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate.

Figure 1 shows the frequency distribution of the $H - F$ differences in

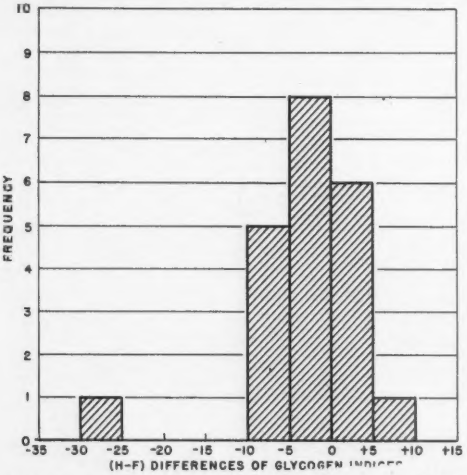


FIGURE 1.—Histogram showing frequency distribution of hind-fore end differences of 21 ligatured control larvae.

glycogen index for the group of 21 ligatured control larvae, when the class intervals are as shown in the figure. The −25 to −30 value was for a larva having H and F glycogen indices of 60.75 and 86.75 percent, respectively.

Most of the unligatured larvae constituted groups less suitable for statistical comparison. The mean glycogen index and the standard deviation for the 23 unligatured larvae subjected to nicotine vapor for various periods up to 29.5 hours were 42.61 and 14.24 percent, respectively; the corresponding values for the 5 unligatured controls were 50.00 and 11.81 percent. The difference between these mean glycogen indices is 7.39, and the standard error of this difference is 6.06. These values indicate that the larvae treated with nicotine vapor did not differ significantly from their controls, although some decrease in glycogen index might follow very prolonged treatment.

The values given in table 2 show the relationship between glycogen index and stage of general hematological change subsequent to administration of each of the effective poisons. Each hind-end value is an average glycogen index of the hind ends of all the ligatured larvae that yielded the corresponding fore-end values. The fore-end values are averages of the fore-end glycogen indices grouped according to

whether they showed 0, +, ++, or +++ general hematological change. These values indicate that the glycogen index tends to undergo a progressive decrease as the general hematological changes proceed, and that usually the most marked reductions of glycogen index occur during the ++ and +++ stages.

TABLE 2.—*Average glycogen indices corresponding to progressive stages of general hematological changes following poison administration to ligatured larvae of Prodenia eridania*

Poison	Glycogen index of—				
	Hind end	Fore end with indicated stage of hematological change			
		0	+	++	+++
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Barium fluosilicate	32.90	34.00	19.50	16.56	7.00
Sodium fluoaluminate	36.70	39.00	----------	14.25	7.16
Sodium fluoride	42.54	36.50	40.33	19.00	13.25
Mercuric chloride	35.82	32.31	28.25	18.25	11.70
Calcium arsenate	52.60	----------	27.63	14.42	----------
Calcium arsenite	27.10	----------	----------	----------	1.70
Arsenic trioxide	49.62	55.80	41.25	32.19	15.05
Paris green	37.66	35.38	30.63	9.42	1.00
Lead arsenate	41.35	57.88	50.58	15.25	6.85

GLYCOGEN COUNTS

The average glycogen counts of all the nonligatured larvae and of the hind ends plus fore ends of the ligatured control larvae are presented graphically in figure 2, *A*. It is shown that the curves from nonpoisoned larvae tend to have the form $0>1>2>3<3+$, where the numbers represent classes of the count and the symbols indicate their numerical relationship.[3] Figure 2, *B*, shows that the average

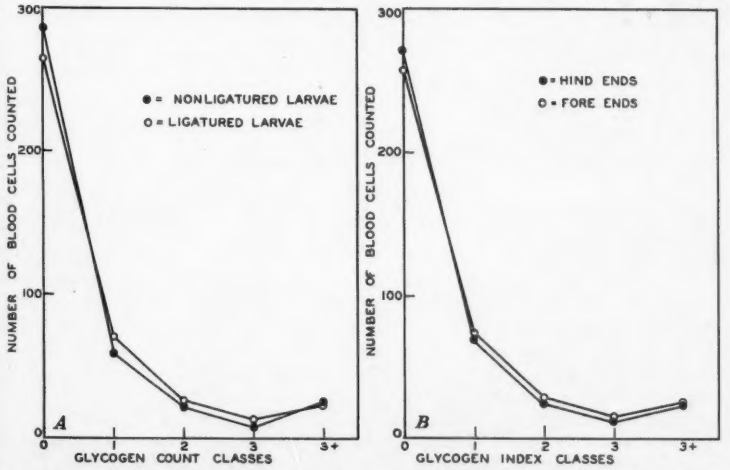


FIGURE 2.—Average glycogen counts from larvae fed turnip-leaf sandwiches containing carbohydrate but no poison (controls): *A*, Nonligatured and ligatured larvae; *B*, Hind and fore ends of ligatured larvae.

[3] A discussion of the form of glycogen counts from unpoisoned larvae has been given in a previous paper (*31*).

glycogen counts of hind and fore ends are similar in form and that the counts from the fore ends may tend to exceed those from the hind ends in classes 1 to 3+, inclusive.

Average glycogen counts of the hind and fore ends of ligatured larvae given nicotine bentonite, nicotine peat, rotenone, and phenothiazine are shown in figure 3. All these counts have the usual $0>1>2>3<3+$ form. Unlike the corresponding curves of the controls (fig. 2, *B*), the hind-end and fore-end curves cross somewhere between classes 1 and 3+. Average glycogen counts of the hind ends, the fore ends that showed no definite general hematological change,

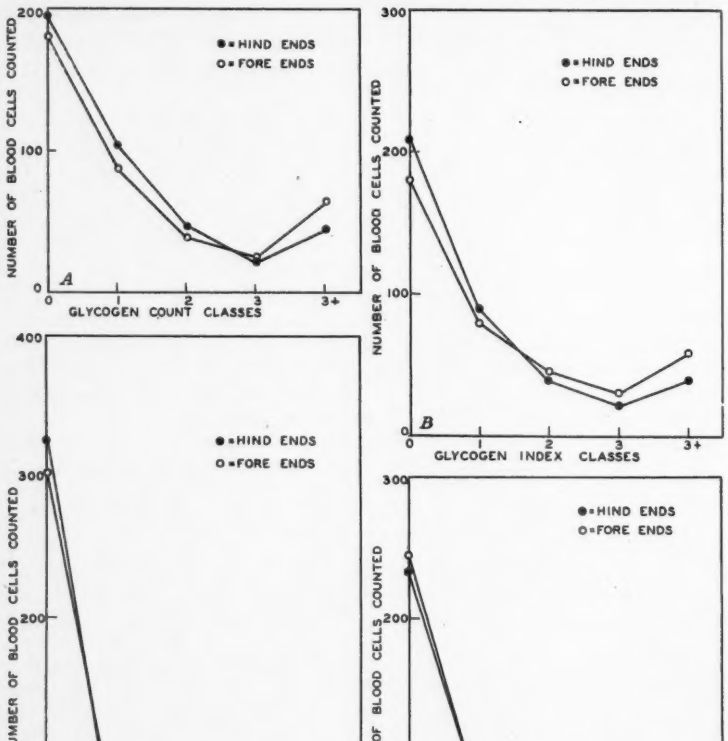


FIGURE 3.—Average glycogen counts from the hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then fed cornstarch-turnip-leaf sandwiches containing the following poisons: *A*, Nicotine bentonite; *B*, nicotine peat; *C*, rotenone; *D*, phenothiazine.

and the fore ends that showed a + stage of general hematological change subsequent to pyrethrum administration are presented in figure 4. Crossing between classes 1 and 3+ also occurs in this figure.

A comparison of average glycogen counts (not illustrated) for the combined hind ends and for the combined fore ends of all the larvae given calcium arsenate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate showed that the hind ends tended to exceed the fore ends in classes 1 through 3+. The corresponding average glycogen indices were 42.88 and 25.65 percent.

Figure 5 contains the average glycogen-count curves for the combined hind ends and for the fore ends grouped according to the type

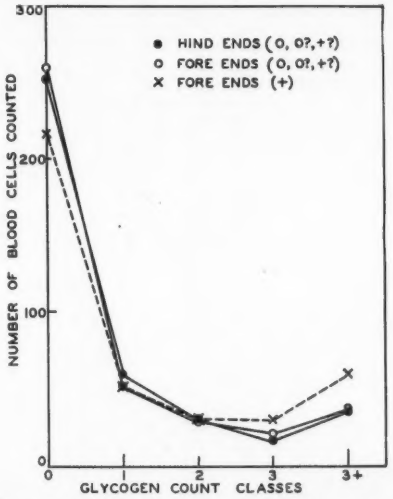


FIGURE 4.—Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given pyrethrum, or pyrethrum in cornstarch-turnip-leaf sandwiches. The fore ends are separated into those that showed no effect or a questionable + effect and those that definitely showed a + general hematological effect of poison administration.

of hematological change. The curves indicate that, relative to their hind ends, the 0 fore ends tended to have a somewhat larger number of glycogen inclusions, but that the +, ++, and +++ fore ends contained progressively smaller numbers of glycogen inclusions. The + and ++ fore-end curves are separated by a relatively large interval. Because the curves in *A* are considered to represent in general the effects of administration of each of the arsenical poisons used in this work, the curves for each poison are not presented. The curves for paris green (*B*), however, indicate a progressive decrease in number of blood-cell glycogen inclusions as the general hematological changes progress to the +++ stage.

The larvae receiving calcium arsenite had fore ends showing only +++ conditions and hind ends in which slight hematological changes tending toward the + condition usually seemed to occur. One larva had a hind end showing a ++ condition. The

larvae given calcium arsenate had hind ends showing 0 and fore ends showing only + and ++ changes.

The glycogen counts for larvae given arsenic trioxide and lead arsenate present a graphical picture similar to those of figure 5.

A comparison of average hind-end and fore-end counts (not illustrated) for barium fluosilicate, sodium fluoaluminate, and sodium fluoride showed that, as was true for the arsenical curves, the numbers of blood-cell glycogen inclusions in classes 1 to 3+ for the hind ends tended to exceed those for the fore ends. Figure 6, *A*, corresponding

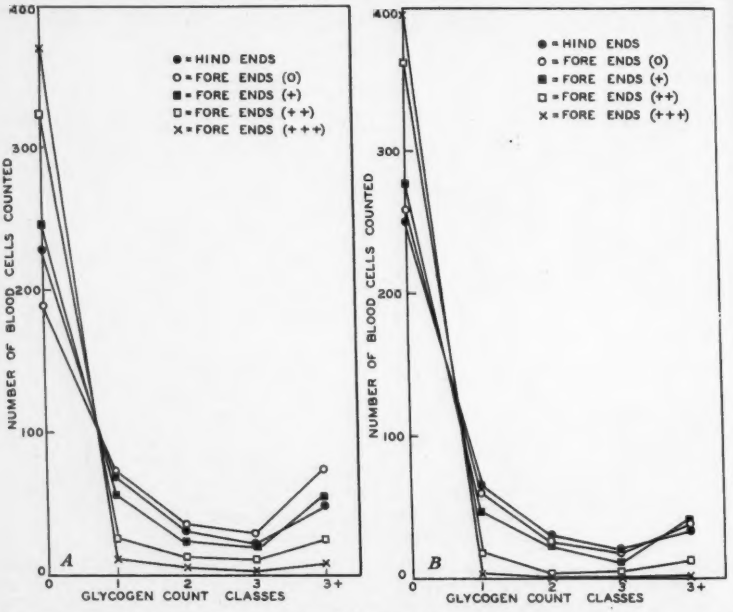


FIGURE 5.—Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given poison in cornstarch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, ++, or +++ stage of hematological change: *A*, Larvae poisoned with Paris green, lead arsenate, calcium arsenite, calcium arsenate, and arsenic trioxide; *B*, larvae poisoned with paris green.

to figure 5, *A*, shows the average glycogen curves for the combined hind ends and for the combined 0, +, ++, and +++ fore ends of the larvae given these poisons. Here also the hind-end and 0 and + fore-end curves, and the ++ and +++ fore-end curves tend to fall into two groups, which are separated by a relatively large interval. Among the curves for the individual poisons, this grouping was most marked when sodium fluoaluminate was used. When barium fluosilicate was used, the grouping was slight and the relatively large interval was between the 0 and + fore-end curves (fig. 6, *B*). These curves indicate a progressive decrease in the number of blood-cell glycogen inclusions of classes 1 to 3+ as the hematological changes progress. Because figure 6 is considered to represent the general

effects of administering the fluoride poisons used in this work, the curves for sodium fluoaluminate and sodium fluoride are not presented.

Figure 7 contains average-glycogen-count curves for the hind ends and for the 0, +, ++, and +++ fore ends of the larvae given mercuric chloride. The curves indicate a progressive decrease in number of blood-cell glycogen inclusions as the hematological changes progress. As with the arsenical and fluoride curves, a tendency toward grouping is shown. The value for the 3+ class is relatively

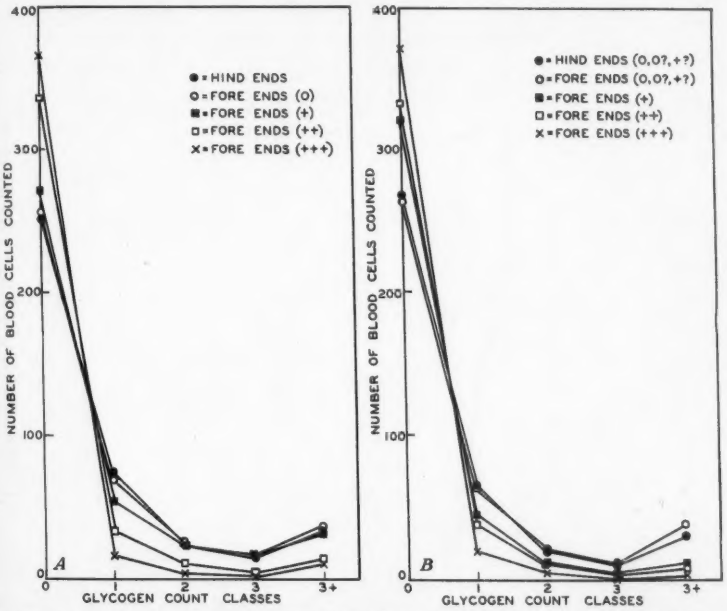


FIGURE 6.—Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given poison in cornstarch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, ++, or +++ stage of hematological change: *A*, Larvae given sodium fluoaluminate, sodium fluoride, and barium fluosilicate; *B*, larvae given barium fluosilicate.

high in the ++ fore-end curve. Similarly, relatively high values of the 3+ class occur in the + fore-end curves of figure 5.

Average glycogen counts for the nonligatured larvae subjected to nicotine vapor for periods up to 29.5 hours and for the corresponding control larvae are represented in figure 8. The two curves are of the same form and show no marked differences.

All the average curves, such as those shown in figures 2 to 8, have the form $0>1>2>3<3+$.

DISCUSSION

METHODS

The complicating factor of individual differences between larvae was eliminated by the use of ligatured insects, because blood from the

fore end could be compared with blood from the hind end of the same individual. Any hematological change resulting from the ingestion of a poison by an adequately ligatured larva can be expected to appear only in blood from its fore end, whereas blood from the hind end may be used as a control. A differential effect upon the blood that might be produced by ligaturing should be apparent from a comparison of fore-end and hind-end blood taken from a ligatured but unpoisoned larva. A poison coming in contact with the integument of either end may be expected to occur in and possibly to affect the blood in that end, provided the integument is permeable to the poison.

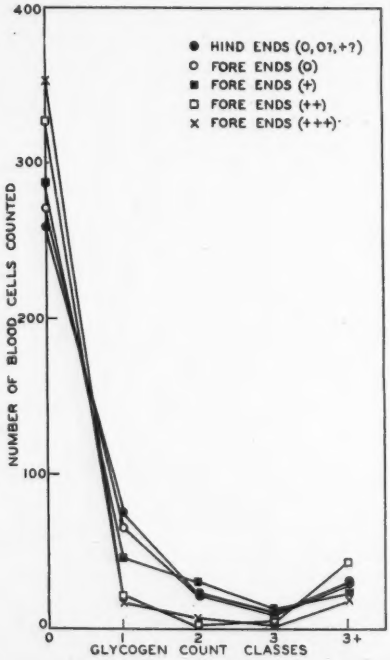


FIGURE 7.—Glycogen counts from the hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given mercuric chloride, or mercuric chloride in cornstarch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, ++, or +++ stage of hematological change. The + and ++ fore-end curves represent single counts; the other curves, average counts.

Several investigators have reported that some insecticides can penetrate the integuments of some insects. For example, integumental absorption of arsenic has been reported by O'Kane and Glover (*17*), Lepesme (*14*), and Woodworth (*27*); of fluoride by Hockenyos (*10*); of pyrethrum by Wilcoxon and Hartzell (*25*); and of nicotine by Richardson, Glover, and Ellisor (*21*). The nicotine vapor possibly entered the insect's body partly by integumental absorption and partly through the tracheal system. Kitchel and Hoskins (*11*) and McGovran (*16*) have shown that in nicotine vapor (of lower concentrations

than used here) tracheal ventilation of the cockroach and of the grasshopper does continue.

In most of the present experiments the larvae were free to bring the integument of either the fore or the hind end in contact with the administered poison, but in several experiments they were placed in stocks (pl. 1, *B*) to prevent contact of the hind end either with the fore end or with the poisoned sandwich. Although some integumental absorption probably occurred, for example, when calcium arsenite was used, it is considered that the poison entered the larva's body chiefly by ingestion and intestinal absorption, because of the occurrence in some of the smears of abnormal midgut epithelial cells, some of which were much vacuolated, large numbers of bacteria, and fragments of partly digested leaf. Parfentjev (*18*), Pilat (*19*), and

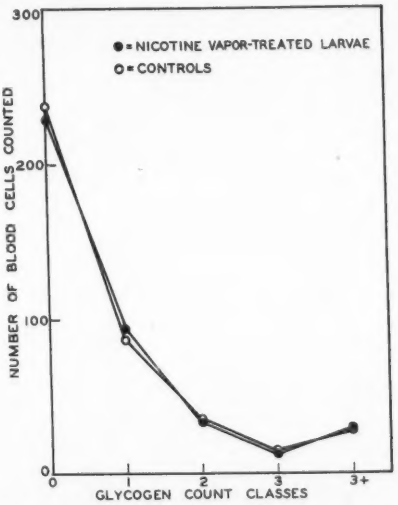


FIGURE 8.—Average glycogen counts from nonligatured larvae fed carbohydrate-turnip-leaf sandwiches and then treated with nicotine vapor for periods up to 29.5 hours, and from their controls.

Woke (*26*) have demonstrated that ingestion of arsenicals and fluorides may be followed by necrosis and loss of midgut epithelium. The poisoned and unpoisoned sandwiches were like those used by Campbell and Filmer (*2*), except that the sandwiches used here contained cornstarch, or cornstarch and sugar, and an unmeasured, relatively large amount of poison. Ligatured insects have been used by Fraenkel (*7*) in an investigation of hormones in *Calliphora* and by Ermakov (*3*) in a study of the reducing power of silkworm blood.

Comparison of blood smears from fore and hind ends of unpoisoned larvae indicated that blood-cell glycogen tended to become relatively lower in the hind end, particularly in larvae that had been ligatured for the longer periods (pl. 3, *F*, and negative value of M_{H-F} in table 1). Although this difference might result from an actual increase in the fore end, a decrease in the hind end, or certain combinations of changes in both ends, it is probably due partly to a real decrease in

the hind end, because microscopic observations indicated that prolonged ligaturing may induce degenerative changes sooner in this end. In some experiments hind-end blood smears showed a + or, in several larvae, even a ++ effect, particularly with calcium arsenite, mercuric chloride, and arsenic trioxide. These poisons must therefore have penetrated into the hind end of the larva either by absorption through the integument or by diffusion from the fore end through the ligatured region.

The glycogen counts and indices are based upon 400 cells taken at random, since this number could be checked satisfactorily for the purpose of this work and it is the same as that recommended by Kolmer and Boerner (*12, p. 85*) and given by Kracke and Garver (*13, p. 480*) for bloods with total counts of 20 to 25 thousand cells per cubic millimeter.[4]

GENERAL HEMATOLOGICAL CHANGES AND PASSIVE-ACTIVE TRANSFORMATION

For a more complete understanding of the initial changes in the blood cells of an insect subsequent to poison administration, it is necessary to consider the form changes that blood cells of many insects undergo under certain physiological conditions. Tait (*23*) and Loeb (*15*) observed several types of blood coagulation in Crustacea. Yeager and Knight (*30*) observed similar kinds of blood coagulation in a number of different insects. Coagulation of insect blood was found to involve a plasma coagulation, a cell coagulation or agglutination, or both. In a study of the coagulation of blood from the oriental cockroach, Yeager, Shull, and Farrar (*32*) found that during this process the blood cells may lose their original fusiform or discoid shape, round up, become more refractive, form pseudopodia, agglutinate into a number of clumps, spread out on supporting surfaces, and seemingly disintegrate. They also reported that heat fixation of the roach at 60° C. for 5 to 10 minutes prevents subsequent coagulation and changes in form of the blood cells. Ermin (*4*) also has recently studied the changes undergone by coagulating insect blood cells.

Marked change in form of blood cells during coagulation has long been recognized as characteristic of invertebrate blood (*8, 9*, and others cited in *5, 4*, and *22*). Unpublished observations by the present authors have shown that coagulation of the blood of *Prodenia eridania* involves marked changes in form of several types of blood cells, including the plasmatocytes and the cystocytes. Thus, at least certain types of insect and other invertebrate blood cells are capable of existing in two different forms. It may be considered that such a blood cell tends to assume one form—for example, a spindle shape—when suspended in the circulating blood plasma in a quiescent state, unaffected by exciting stimuli, but when affected by exciting stimuli to undergo changes such as withdrawing its spindle ends, tending to round into a spheroid or ovoid, producing irregularities in its surface protoplasm, extending some type of pseudopodium or lamella, or tending to form a very thin cell, widely spread upon a surface. Through some such change a cell having a passive form and suspended in the circulating blood plasma may develop an active form and display some kind of cellular activity. Such activity may involve the

[4] Counts made by the senior author and J. B. Gross indicate that the average value for the mature larva of *Prodenia eridania* is about 20 to 25 thousand cells per cubic millimeter of blood.

formation of pseudopodia, with or without actual locomotion, cellular locomotion, capsule formation, possibly phagocytosis, etc.

Fauré-Fremiet (*5*) made an extensive study of passive and active states of blood cells of a number of marine invertebrates other than insects, giving careful descriptions of each state and of the passive-active transformations undergone by various kinds of cells. The present authors have observed insect blood cells, in the active state that are analogous to some of the active forms described by Fauré-Fremiet; these observations were made particularly upon blood from *Prodenia eridania* and *Periplaneta americana*. The senior author has further observed such passive-active transformations and active forms in several other species of insects (*30*).

A study of the plasmatocytes and cystocytes in the blood smears showing a + hematological change obtained from larvae given the arsenicals, the fluorides, and mercuric chloride indicated that one of their earliest changes is from a passive to an active form. In fact, the chief characteristics of the + stage of general hematological change can be explained to a great extent on the basis of passive-active transformation and blood-cell coagulation. The slight tendency of some of the blood cells from larvae given noneffective poisons to deviate from the normal was exhibited chiefly as partial passive-active transformation. The + stage, therefore, is considered to represent a certain physiological state of the blood which might be, but is not necessarily, accompanied by a toxicological condition of the blood cells. This physiological state of the blood is characterized by the partial or complete transformation of many of the plasmatocytes and cystocytes and an increase of blood-cell agglutination in vivo. In smears from presumably normal larvae there is often a slight, variable amount of blood-cell agglutination.

The ++ stage of general hematological change involves the changes characteristic of the + stage, explainable for the most part on a physiological basis and not necessarily toxicological as regards the blood cells. It also involves a more marked agglutination, some distortion, and perhaps some disintegration, all of which, particularly the distortion and disintegration, may represent a toxicological or a pathological disturbance of the affected cells.

The +++ stage differs from the ++ stage chiefly in the greater amount of cell distortion and disintegration, or, in other words, in a greater toxicological disturbance of the blood cells, which frequently appeared in obviously smaller numbers during this stage. The decrease in numbers of cells could result from a complete disintegration of some of the affected cells or from a dropping out of the circulating blood of clumps of agglutinated cells or of single cells. In invertebrate blood coagulation, during which certain cells undergo passive-active transformation, their surfaces undergo physicochemical changes resulting in increased stickiness (*4*, *5*, *15*, *23*, *32*). The cells adhere to one another and tend to form agglutina, which can be caught among various tissues and thus removed from the circulating blood. The active blood cells also tend to adhere to certain surfaces, including those of injured tissues.[5] It is thus quite possible that blood cells disappeared from the circulating blood through cellular degeneration, through adhering to certain injured tissues in

[5] Such adhesion of the blood cells to the tissues at the site of injury can be demonstrated by observing the circulating blood in the wing of a cockroach (*28* and *29*) and thrusting a needle through the wing.

the poisoned portion of the insect's body, and through the removal of cell agglutina from circulating blood to tissues. The observation that blood cells tend to decrease especially in the ++ and +++ stages of general hematological change is in agreement with the report of Fisher (*6*) that a decrease in the total blood-cell count of the oriental cockroach occurs subsequent to the administration of lethal doses of white arsenic, sodium fluosilicate, and mercuric chloride.

RELATION OF GLYCOGEN DECREASE TO GENERAL HEMATOLOGICAL CHANGES

The glycogen-count curves in figures 5 to 8 and the glycogen-index values given in table 2 indicate that, in general, blood-cell glycogen shows little or no decrease in the + stage and the greatest decrease in the ++ and the +++ stages. This observation is in accord with the view that the + stage represents largely a physiological condition of the blood, whereas the ++ and +++ stages represent predominantly toxicological conditions.

CYTOLOGICAL CHANGES FOLLOWING POISON ADMINISTRATION

Possible Regenerative Changes

The only indication of regenerative changes noticed in these experiments was an apparent tendency of the nuclei of some cells to exhibit mitotic, particularly prophasic, aspects. Some of these nuclei were obviously in a mitotic condition, but many were questionably so. The mitotic cells seemed to be unusually numerous, but as the microscopic observations were only qualitative an increase of these cells was not definitely demonstrated. The possibility of the occurrence of such regenerative changes is stressed here because similar observations of an apparent increase in mitosis among blood cells from poisoned insects have been reported by Pilat (*20*), Lepesme (*14*), and Tareeva and Nenjukov (*24*). None of these authors reported quantitative mitotic counts. Further investigation of a more quantitative character appears to be necessary to demonstrate definitely an increased rate of mitosis in insect blood subsequent to poison administration.

Degenerative Changes

Observations on the blood cells from poisoned insects have been made by Pilat, Lepesme, and Tareeva and Nenjukov. Pilat (*20*) studied the blood cells of *Locusta migratoria* that had been poisoned with sodium arsenite and sodium fluosilicate (silicofluoride). Although he considered that poisoning produced degeneration of some blood cells, Pilat concluded that the blood picture of the poisoned insect is too complicated to be quantitatively expressed until precise and uniform principles of blood-cell classification are established. Tareeva and Nenjukov (*24*) reported degeneration among the blood cells of *Calliptamus* given sodium arsenite. Cellular degeneration apparently involved achromophilia, cytoplasmic fragmentation and disintegration, nuclear fragmentation and disintegration, and reduction of nuclear-cytoplasmic differentiation. Lepesme (*14*), in a study of the penetration of arsenicals through the integument of *Schistocerca gregaria* Forsk., found that blood cells from poisoned insects exhibited

cytoplasmic vacuolization, loss of cellular contours, and disintegration of the chromatin masses.

None of these authors studied the effects of poison administration upon the blood cells of Lepidoptera, they used different hematological techniques from those used in the present experiments, and they did not consider the possibility of passive-active transformations among the blood cells that they studied. Their descriptions can therefore be compared only in a general way with observations reported here for *Prodenia eridania*, but there is agreement in indicating that administration of the arsenicals and fluorides can produce degeneration of blood cells in insects.

With regard to degenerative changes, the present observations on the blood of poisoned larvae of *Prodenia eridania* show not only that different types of cells may respond differently to the administration of one of the effective poisons, but also that the arsenicals, the fluorides, and mercuric chloride may produce somewhat different effects upon a given type of blood cell. They also indicate that blood-cell degeneration involves several kinds of cytological change, among which are plastid formation, achromophilia, partial or complete loss of cytoplasmic structure, disappearance of glycogen inclusions (with or without an accompanying diffuse glycogen stain (Bauer)), disruption of nuclear structure, and nuclear extrusion. Plastid formation may be a manifestation of a pronounced or abnormal pseudopodial activity. The disappearance of cytoplasmic structures and the presence of a diffuse glycogen stain coincident with the disappearance of glycogen inclusions may indicate a dissolution of these structures and of glycogen.

Effects of Administration of Different Poisons on Cystocytes and Plasmatocytes

The scheme shown in figure 9 has been devised to illustrate in a general way the changes undergone by the plasmatocytes and the cystocytes subsequent to the administration of the effective poisons. It indicates that during the + stage of general hematological change the affected plasmatocyte or cystocyte tends to change from its passive to its active form. In their active form some of these cells possess smooth surfaces and may appear swollen, while others may not appear swollen but may exhibit various surface irregularities, involving the ectoplasm. Thus far these changes can be considered to be physiological rather than toxicological, with the possible exception of the swollen aspect, which may be abnormal. The questionable occurrence of regenerative, mitotic changes during the + or ++ stage is indicated. To what extent these changes and the final assumption of all the different cellular forms that have been called active can be considered to be physiological or toxicological is problematical. The degenerative changes, which may be considered toxicological, occur chiefly in the ++ and +++ stages. During these changes blood-cell glycogen decreases or disappears. Cellular degeneration may or may not involve plastid formation. The various details previously presented and discussed are implied by this scheme, but they have not been actually represented.

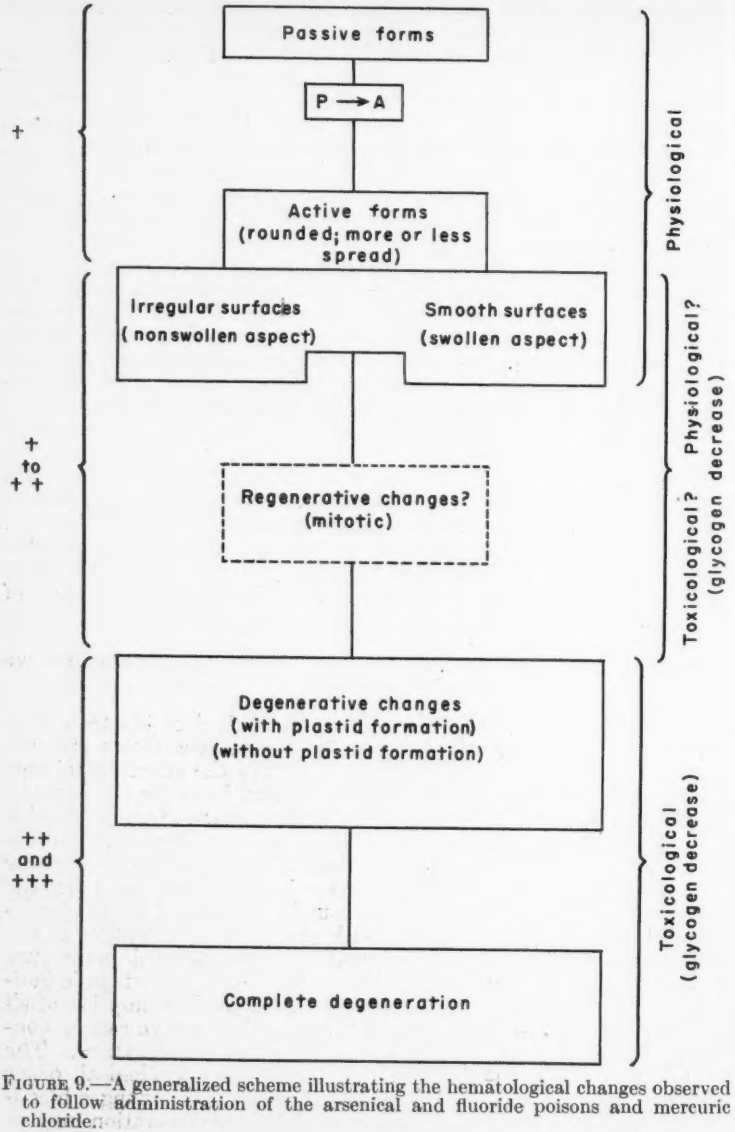


FIGURE 9.—A generalized scheme illustrating the hematological changes observed to follow administration of the arsenical and fluoride poisons and mercuric chloride.

POSSIBLE CHANGES AFTER ADMINISTRATION OF THE OTHER POISONS

Although no obvious cytological changes followed administration of nicotine peat, nicotine bentonite, and rotenone, occasional blood cells looked as though they might be just beginning to undergo pas-

sive-active transformation. Exposure to nicotine vapor up to 6 hours was followed by no obvious alteration of the blood cells, except perhaps a slight tendency of some of them to round up, but after exposure for prolonged periods (23 hours or more) the blood cells showed a definite tendency toward passive-active transformation and an abnormal amount of vacuolization (pl. 2, *B*). Whether these changes were produced directly by the nicotine vapor or by an accompanying alteration in the insect's body, such as dehydration, was not determined. The fact that the dehydrated controls did not show the same degree of change suggested that with these long exposures the blood cells were somewhat affected, either directly or indirectly, by nicotine vapor. The failure of nicotine vapor readily to produce marked changes in the blood cells is in agreement with the results of Babers (*1*), who similarly exposed *Prodenia eridania* larvae to concentrated nicotine vapor and found that the pH of the blood was maintained at the normal value of approximately 6.65. When pyrethrum was used, the blood cells showed some indications of passive-active transformation. In general, with these exceptions, administration of the noneffective poisons produced no obvious cytological changes in the blood cells.

MECHANISM OF POISON EFFECT

Reference has been made to the effects of poison administration rather than to the effects of poison on blood cells. Although it is probable that at least those degenerative cytological changes and the blood-cell glycogen decrease occurring in the ++ and +++ stages were caused by direct action of the poison in the plasma, proof of this occurrence is not given by the results of this investigation. These results do indicate that administration of the effective poisons caused a decrease of blood-cell glycogen as a part of a general cellular degeneration rather than in a specific manner.

SUMMARY AND CONCLUSIONS

Ligatured larvae whose blood-cell glycogen had previously been increased by ingestion of turnip-leaf-cornstarch-glucose sandwiches were given access to turnip-leaf-cornstarch sandwiches without poison (controls) and turnip-leaf-cornstarch sandwiches containing poison. The poisons used were nicotine bentonite, nicotine peat, rotenone, pyrethrum, phenothiazine, calcium arsenite, calcium arsenate, arsenic trioxide, paris green, lead arsenate, sodium fluoride, sodium fluoaluminate, barium fluosilicate, and mercuric chloride. Blood smears from the fore and hind ends of the poisoned ligatured larvae were stained for glycogen. From a study of these smears results were obtained leading to the following conclusions:

(1) No marked hematological changes followed the administration of nicotine bentonite, nicotine peat, rotenone, pyrethrum, and phenothiazine, although some of the fore-end blood smears from larvae given pyrethrum showed slight changes consisting largely of partial passive-active transformation. To what extent this lack of effect was caused by failure of the administered poison to enter the blood stream was not determined.

(2) Administration of none of the poisons named under (1) caused a significant decrease of mean glycogen index of the fore ends relative to that of the hind ends of the treated larvae, but relative decreases

in fore-end glycogen indices did occur subsequent to administration of the arsenicals, the fluorides, and mercuric chloride.

(3) Marked hematological changes in fore ends relative to hind ends of the ligatured larvae followed the administration of the arsenicals, the fluorides, and the mercuric chloride.

(4) The hematological changes that followed poison administration occurred progressively and, for purposes of description, are grouped according to successive stages as 0, +, ++, and +++, where 0 presents the normal and +++ the stage of most marked change. The + stage is characterized chiefly by partial or complete passive-active transformations (especially rounding up) of certain blood cells, including the cystocytes and the plasmatocytes, and by a slightly augmented cell agglutination. It is thus considered to represent chiefly physiological changes, which may or may not be accompanied by the beginning of certain toxicological changes. The ++ and +++ stages are characterized chiefly by more marked agglutination, distortion, disintegration, and apparent loss of cells from the blood. In general, subsequent to the administration of the arsenicals, the fluorides, and mercuric chloride, the earlier blood-cell changes (+ stage) tended to be chiefly physiological, such as may occur in larvae not subjected to poison administration, whereas the later changes tended to be chiefly toxicological (or pathological).

(5) An increase of mitosis seemed to occur soon after administration of the arsenicals, the fluorides, and mercuric chloride, but was not demonstrated quantitatively.

(6) The degenerative cytoplasmic changes consisted of apparent cellular swelling, disruption of and decrease in visibility of normal structure, achromophilia, decrease or loss of blood-cell glycogen, formation of broad pseudopodia or cytoplasmic bulges, plastid formation, excessive vacuolization, and raggedness. Nuclear degeneration involved distortion, raggedness, loss or disruption of normal structure, achromophilia, assumption of more or less peripheral position, fragmentation, pycnosis, and extrusion.

(7) Subsequent to mercuric chloride administration the nuclei of certain cystocytelike cells gave a positive reaction to the Bauer test for polysaccharide; this reaction was not observed after the administration of the other poisons.

(8) Plastid formation tended to follow arsenical and mercuric chloride administration more readily than fluoride administration.

(9) Abnormal cellular vacuolization (gross vacuoles) tended to follow administration of the fluorides and, especially, of mercuric chloride.

(10) Decreases of glycogen index subsequent to arsenical, fluoride, and mercuric chloride administration tended to occur to a greater extent during the ++ and +++ stages of general hematological change than during the + stage. Thus, the decrease of blood-cell glycogen is considered to occur as a part of cytological degeneration and not as a specific, exclusive effect of poison administration.

(11) As blood-cell glycogen decreased subsequent to poison administration, the glycogen count tended to show a general decrease in the classes 1 to 3+, inclusive, and to maintain the normal form of $0>1>2>3<3+$.

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THE RELATION OF SHANK COLOR TO WINTER EGG PRODUCTION CHARACTERISTICS OF SINGLE-COMB WHITE LEGHORN PULLETS[1]

By I. MICHAEL LERNER

Assistant poultry husbandman, California Agricultural Experiment Station[2]

INTRODUCTION

The belief that heavy egg production is accompanied by the bleaching of pigmented structures in yellow-skinned breeds had been held by practical poultrymen long before the first quantitative data on this correlation were presented by Blakeslee and Warner (*2*, *3*).[3] A series of experiments led Palmer and Kempster (*10*) to suggest that xanthophyll from the feed, instead of being deposited in the ear lobes, beak, shank, eye ring, and the skin surrounding the vent, as it is in males and in nonlaying females, is diverted in laying birds to the forming ova. The epidermal xanthophyll in the meantime "gradually disappears as the result of the natural physiological change in the structure of the skin." Cessation of production is followed by return of xanthophyll to the parts named. These phenomena have enabled poultrymen to estimate the past production of birds from the degree of bleaching or the extent of return of pigment to these tissues.

The quantitative data on the relation between pigmentation and past production presented by various investigators seem to be largely confined to those gathered toward the end of the laying year. Thus Blakeslee and his collaborators (*1*, *4*) found the correlation coefficients between pigment in the ear lobe (determined by matching with a color top) in October of the second year of life and the annual production in two groups of White Leghorns to be -0.5816 ± 0.0253 and -0.5271 ± 0.0252, respectively. By analyzing the relationship of the pigmentation observed in October with the monthly production in the course of the previous year, these investigators concluded that the correlation was largely due to the production during the month immediately preceding the date of observation. Warner (*13*) extended this study to include pigmentation changes in the color of the vent. He also noted that the correlation exists not only at the end of the year, but also at the beginning and middle of the year. Sherwood (*11*), who made observations in September, likewise found his subjective grading of shank and beak color to be correlated with annual production, the coefficients of correlation being of the magnitude of -0.6. Similar results on the basis of subjective color determinations were obtained by Hervey (*5*) and by Knox and Quinn (*7*).

In a recent textbook, Jull (*6*, *p. 431*) suggested that observations on the degree of bleaching of the beak and shank in December or January of the first year of life make it possible to estimate the in-

[1] Received for publication June 10, 1941. Assistance in the calculation of statistical constants was provided by the Work Projects Administration, official project A. P. No. 65-1-08-91-B6.

[2] The writer gratefully acknowledges the aid of A. A. Klose who prepared the comparator used in obtaining the color measurements and of C. A. Gunns who mounted it.

[3] Reference is made by number [italic] to Literature Cited, p. 338.

herent rate of production of individuals thus examined. If the theory of Palmer and Kempster (*10*) is valid, however, pigmentation changes at that time may reflect not only the rate of production but also the length of the preceding laying period, and thus include maturity and winter pausing. Since no work has been reported on the relation of pigmentation changes to the particulate factors of egg production, it appears to be desirable to examine whether the bleaching of the pigmented structures of yellow-skinned pullets depends on the length of the preceding laying period, on the rate of production, or on both.

MATERIAL AND METHODS

The University of California flock of Single-Comb White Leghorn pullets, consisting of birds bred for production as well as for other characteristics, was used for this analysis. All of the pullets alive at the time the observations were made were used. The shank was the only pigmented structure considered.

TABLE 1.—*Composition of standards used in determining shank color*

Shank color grade	Percentage composition		
	Plaster of paris	Chrome yellow	
		Intermediate orange grade	Yellow grade
1	95	5.0	0.0
2	90	10.0	.0
3	85	15.0	.0
4	80	17.5	2.5
5	75	20.0	5.0
6	70	22.5	7.5
7	65	25.0	10.0
8	60	27.5	12.5
9	55	30.0	15.0
10	45	32.5	22.5

In order to obtain objective measurements of color, a color comparator was used. The comparator consisted of a series of 10 test tubes mounted on a wooden block and containing a mixture of plaster of paris and chrome yellow in different proportions. The changes in the value of the mixture from grade to grade were linear in order, so far as the percentage of white was concerned, except for the transition from grade 9 to grade 10. The range of hue and purity used in the comparator were based on a study of the color constants of actual shanks. Table 1 presents the composition of the standards. The colors used approximate those represented in the upper half of plate 9 of the color dictionary of Maerz and Paul (*9*), running from 2 to 6 on the horizontal and from C to L on the vertical scale. The upper third of the back of the shank of each bird studied was matched against the standards under comparable light conditions for all the birds.

The grade was determined jointly by the writer and H. B. Muggle-stone, who assisted in making the observations. The degree of reliability of the grades assigned may be judged by the fact that in a preliminary test on 26 birds, the independent replicate grades showed a correlation coefficient of 0.937 ± 0.024 with the original grades. Birds

were weighed at the same time that shank-color determinations were made. To eliminate age as a variable, the 4 hatches of Single-Comb White Leghorn pullets used in the study were examined about a week apart. These birds were hatched in March and April 1940, and the color and weight observations were made between November 26 and December 17, at about 8 months of age. The total number of birds involved was 969. Of these, 39 had not started laying at the time the observations were made. Their mean shank-color grade was 6.62 ± 0.48, as compared with 3.85 ± 0.07 for the remaining 930 birds. Since production characteristics for these 39 birds were not available, they were omitted from the part of the analysis in which egg-production characteristics were employed.

The color of the shanks preceding initiation of laying of the 930 pullets used in this analysis was not available. Since considerable variation undoubtedly existed with respect to the original intensity of pigmentation in these birds, it may be argued that the observations recorded here do not reflect pigmentation changes but rather represent shank color at the given age. In practice, however, such single examination is the procedure used for evaluation of past production as indicated by the cited suggestion of Jull. Hence, while the precision of the term "pigmentation changes" may be debatable, the observations made nevertheless permit the estimation of the variation in shank color and indirectly in the degree of bleaching.

The present study is not concerned with the nutritional aspects of the problem. It should be noted, however, that the birds of each hatch were divided evenly into two groups at the time of hatching, one group being fed a ration containing about 50 percent of yellow corn, the other a ration in which about 30 percent of barley was substituted for the equivalent amount of corn. When the birds were 5 months of age the second of the rations mentioned was fed to both groups. Fresh greens were supplied approximately every other day to all the birds.

DEFINITION OF TERMS

For the purposes of the following discussion, the terms to be used are defined as follows:

Shank color.—The colorimetric grade assigned to each bird in accordance with the procedure outlined above.

Maturity.—The number of days from hatch to date of the first egg.

Production.—The number of eggs laid by each bird from the first egg to the date on which observations on shank color were made. This is roughly the production to 8 months of age.

Length of laying period.—The number of days elapsed from the date of first egg to the date of shank-color observations.

Days in pause.—The cumulative length of pauses, each period of 7 or more consecutive nonlaying days after maturity being considered a pause.

Days in production.—The length of laying period less days in pause.

Body weight.—The weight of the bird in grams on the day of shank-color observations (roughly at 8 months of age).

Gross rate.—The production times 100 divided by days in production.

Net rate.—The production times 100 divided by active laying days. The distinction between gross and net rate has been discussed in detail by Lerner and Taylor (*8*).

DATA AND DISCUSSION

The means and standard errors of the variables studied are presented in table 2. Although the birds in the different hatches were

examined at approximately the same age, the shank color showed an increase from the earliest to the latest hatch. This progression is not linear. It may be associated with the later maturity of the birds hatched later in the season. The seasonal factor is also in evidence with respect to body weight, the later hatched birds being smaller than the earlier hatched ones at the same age. It is likely that the definitive adult weight has been approached more closely by 8 months of age in the earlier hatches.

TABLE 2.—*Means and standard errors of the variables studied* [1]

Variable studied	Hatch No. 1; 217 birds	Hatch No. 2; 217 birds	Hatch No. 3; 210 birds	Hatch No. 4; 286 birds	All hatches. 930 birds
Shank color.....grade	3.17±0.13	3.60±0.15	3.60±0.13	4.73±0.15	3.85±0.07
Production.....eggs	51.6±1.5	50.6±1.6	52.2±1.5	45.7±1.3	49.7±0.7
Length of laying period.....days	81.3±1.8	84.7±1.8	80.3±1.6	76.1±1.5	80.3±0.8
Days in production.....do	70.6±1.9	72.1±1.9	73.2±1.8	65.2±1.7	69.9±0.9
Body weight.....grams	1,719±14	1,658±16	1,660±16	1,601±14	1,655±8
Maturity.....days	169.4±1.7	172.1±1.7	173.5±1.6	178.6±1.5	173.8±0.8
Net rate.....percent	71.8±0.8	68.5±0.8	70.7±0.8	70.0±0.8	70.2±0.4
Gross rate.....do	63.7±1.3	59.4±1.3	64.5±1.3	59.8±1.2	61.7±0.7
Days in pause.....days	10.7±1.4	12.6±1.4	7.1±1.1	10.9±1.3	10.4±0.6

[1] The slight discrepancies between the actual derived variables (days in production, net and gross rate) and the various ratios of the primary variables (production, length of laying period, days in pause) which lead to the calculation of the derived variables from the tabular means, are due to rounding off of decimals in the calculations.

Table 3 presents the coefficients of correlation between shank color and the other variables studied for each hatch separately, and for the group as a whole. The latter coefficients are shown as computed from the ungrouped population (observed) and as obtained by the z, transformation (calculated) from the individual values in the four hatches (*12*). The closeness of the observed and the calculated values would suggest that the former can be safely used as the best estimate of the magnitude of the correlation in the population as a whole. There are two possible departures from this: (1) The correlation of shank color with body weight increased significantly from the first to the fourth hatch, and (2) the correlation with net rate was lower in the last two than in the first two hatches. It is doubtful whether any significant conclusion can be derived from the latter decrease. So far as the shank color-body weight relationship is concerned, it is possible that the preproduction correlation between these two variables is high and is gradually reduced as the shank color bleaches.

TABLE 3.—*Coefficients of correlation of shank color with variables indicated*

Variable	Hatch No. 1	Hatch No. 2	Hatch No. 3	Hatch No. 4	All hatches	
					Observed	Calculated
Egg production	−0.503	−0.612	−0.548	−0.463	−0.529	−0.53
Length of laying period	−.241	−.390	−.298	−.313	−.326	−.31
Days in production	−.479	−.611	−.518	−.480	−.521	−.52
Body weight	−.055	.026	.184	.549	.173	.19
Maturity	.235	.381	.287	.305	.328	.31
Net rate	−.339	−.352	−.179	−.069	−.209	−.24
Gross rate	−.480	−.471	−.494	−.353	−.432	−.45
Days in pause	.375	.359	.407	.284	.329	.36
r at $P=0.05$	.138	.138	.138	.113		
r at $P=0.01$	.181	.181	.181	.148		

Thus the shank color in hatches 1 and 2 is independent of body weight, whereas a high correlation is still shown in hatch 4.

The highest correlation coefficients observed were between shank color on the one hand and egg production and days in production on the other. Since these two variables are themselves highly correlated, it is possible that one or the other is only indirectly related to shank color. An analysis of partical correlation coefficients, which are presented in table 4, yields an answer to this question.

TABLE 4.—*Coefficients of partial correlation between shank color and the variables indicated*

Variable correlated with shank color	Variables eliminated	Hatch No. 1	Hatch No. 2	Hatch No. 3	Hatch No. 4	All hatches
Egg production	Length of laying period	−0.472	−0.514	−0.492	−0.359	−0.442
	Body weight	−.507	−.619	−.581	−.617	−.554
	Maturity	−.477	−.520	−.509	−.365	−.441
	Net rate	−.440	−.536	−.533	−.464	−.498
	Days in pause	−.394	−.539	−.439	−.385	−.447
Length of laying period	Egg production	.144	.037	.115	−.009	.045
Maturity	do	−.154	−.041	−.173	.013	−.041
Net rate	do	−.097	.017	.099	.080	.040
Days in pause	do	.171	.102	.194	.064	.096
Egg production	Maturity, Net rate, Days in pause	.104	−.003	−.184	.112	−.034

It may be seen that when the effect of egg production is eliminated, the length of the laying period is no longer significantly correlated with shank color. Under the same condition shank color is not significantly correlated with days in pause.[4] Since the days in production are obtained by the subtraction of days in pause from the length of laying period it may be seen that egg production is the primary correlative of shank color.[5] However, egg production itself is determined by maturity, net rate, and days in pause. When the effect of any one of these is eliminated, egg production still shows a high correlation with shank color. Conversely, the elimination of egg production in the partial correlation of each of these variables with shank color reduces the respective coefficients to nonsignificant levels. Yet the third-order partial correlation between egg production and shank color, with the effects of maturity, net rate, and days in pause eliminated is practically zero. It thus appears that the combined action of these three factors acting through egg production is the significant determinant of the changes in shank color. Individually, their contribution is low; combined they account for 27.98 percent of the variance in shank color. The multiple determination of shank color, with egg production and body weight as the independent variables, increases this figure to 32.74 percent.

This, of course, means that shank color as a criterion of production cannot be used for independent estimation of the component factors of egg production, such as rate, as suggested by Jull (*6*). It

[4] It may be noted in passing that once more no correlation was found between net rate and length of winter pause. The correlation coefficient for the combined population was −0.091, confirming the suggested independence of these variables (*8*).

[5] It is possible that the significant factor determining depth of pigmentation is the length of time that the birds are in production without pauses of sufficient length to permit deposition of pigment. If this is true, then rate would have no effect unless birds are capable of laying very slowly and at the same time deposit pigment in the shanks. There is, however, no evidence that such is the case. It is unfortunate that because of the intimacy of the correlation between production and days in production this alternative cannot be subjected to verification. Should this idea be valid it only lends further support to the main conclusion that rate of production cannot be evaluated by pigmentation changes.

is an exceedingly valuable tool in culling practice for identification of low or nonproducing individuals. In flock-testing procedures, however, observations on shank color cannot replace trap-nesting, if information on the component factors contributing to the egg record is desired.

SUMMARY

An analysis of the relation of shank color at 8 months of age to production factors in a flock of 930 Single-Comb White Leghorns indicates that:

(1) Differences in egg production account for about 28 percent of the variance in shank color.

(2) This relationship is due to the combined effect of maturity, rate, and pause, rather than to any of these variables alone.

(3) Because of this combined effect, pigmentation changes cannot be used as a criterion of measurement of differences between pullets in the component factors contributing to the egg record.

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RATE AND DATE OF SEEDING KANRED WINTER WHEAT AND THE RELATION OF SEEDING DATE TO DRY-LAND FOOT ROT AT AKRON, COLO.[1]

By D. W. ROBERTSON, *agronomist*, O. H. COLEMAN, *assistant agronomist, Colorado Agricultural Experiment Station*, J. F. BRANDON, *associate agronomist, Division of Dry Land Agriculture*, H. FELLOWS, *associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry*, and J. J. CURTIS, *assistant agricultural technologist, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture*

INTRODUCTION

Experiments to ascertain the best rate and date to seed winter wheat have been conducted in the Great Plains area for various periods since the establishment of experiment stations in that area. Martin [2] summarized the results of investigations conducted in the Great Plains and Great Basin to 1925 as follows:

> The optimum rate of seeding for wheat is practically independent of soil type, moisture, locality, date of seeding, cultural treatment, and variety. The optimum date of seeding for winter wheat is independent of soil type, annual precipitation, variety, and rate of seeding, but is related somewhat to temperature, even though the same temperature conditions do not apply in all sections. Rates of 4 to 6 pecks per acre in general have produced the highest net yields of winter and spring wheats. Medium seeding of winter wheat usually is most favorable.

The results of two experiments, one started in 1920 to ascertain the best rate and date of seeding Kanred (C. I. 5146) winter wheat (*Triticum aestivum* L.) and a second one started in 1930 by H. Fellows to study the occurrence and severity of dry-land foot rot as affected by the date of seeding, will be discussed in this paper. The studies were conducted under dry-land conditions at the United States Dry Land Field Station, Akron,[3] Colo., where the average annual rainfall is approximately 17 inches.

EXPERIMENTAL METHODS

The soil of the Akron station is classified as Weld silt loam. It is a light brown loam underlaid at a depth of 12 to 15 inches by a calcareous layer. The subsoil is slightly heavier in texture than the surface soil.

In the studies of date and rate of seeding, plots were seeded on or near the following dates during the period from 1920 to 1937: August 15, September 1, September 15, October 1, and October 15. In 1938

[1] Received for publication June 2, 1941. Paper No. 122, Scientific Journal Series, Colorado Agricultural Experiment Station.

[2] MARTIN, JOHN H. FACTORS INFLUENCING RESULTS FROM RATE- AND DATE-OF-SEEDING EXPERIMENTS WITH WHEAT IN THE WESTERN UNITED STATES. Amer. Soc. of Agron. Jour. 18: 193-225, illus. 1926.

[3] The United States Dry Land Field Station, located in northeastern Colorado at an altitude of about 4,600 feet, is operated by the Division of Dry Land Agriculture of the U. S. Department of Agriculture, in full cooperation with the Colorado Experiment Station. The cereal experiments at this station were under the care of a representative of the Division of Cereal Crops and Diseases of the U. S. Department of Agriculture, also in cooperation with the Colorado Experiment Station. The rate-and-date experiment was carried on at this station by the representative of the Division of Cereal Crops and Diseases from 1920 to 1924, by J. F. Brandon and D. W. Robertson from 1924 to 1930, and by J. J. Curtis from 1930 to August 1940.

and 1939, plantings were made weekly between August 15 and October 1. The wheat was seeded at the following per-acre rate on each date: 1 peck, 2 pecks, 3 pecks, 4 pecks, and 5 pecks, except in 1926, 1927, and 1928, when the 6-peck rate was used instead of the 1-peck rate. Four replications of each rate on each date were sown, two on fallow and two on cornland

From 1920 to 1925 all plantings were made with an 8-inch disk drill. After 1925 a 12-inch furrow drill was used. From 1920 to 1925 the plots were one-forty-fifth of an acre in size. After 1925 smaller plots, one-fifty-fifth of an acre, were used.

Dry-land foot rot occurs throughout the semiarid region of the United States. Its presence is noticeable in the early planted fields of winter wheat. The appearance of a moderately diseased field

FIGURE 1.—Winter wheat showing normal plants (*A*) and plants infected with dry-land foot rot (*B*).

resembles that of a field that has been subjected to extreme drought. The plants ripen prematurely and are more or less stunted. The wheat in the diseased portion of a field when viewed from a distance is more or less ashy gray in color. In a badly diseased field the plants may die before heading occurs.

The study of the relationship of dry-land foot rot to date of seeding was made on summer fallow. An additional set of plots seeded at 3 pecks per acre was planted at each date in the rate-and-date study.

In studying the amount and severity of foot rot, plants from 5 feet of row taken at random were pulled for each date of planting at or shortly before ripening and the crowns were examined for the presence and severity of foot-rot lesions. The crowns were cut the long way (fig. 1). The severity was determined mainly by the

extent and darkness of the lesions, and in part by the progress of decay in the crowns.

Analysis of variance [4] was used in the interpretation of the data. Snedecor's [5] F value was used to determine the significant departures.

The homogeneity tests [6] of the yield data of years for fallow and cornland indicate that the data for the various years cannot be considered to have been drawn from the same general population. Thus, the high value of χ^2 indicates that a valid determination cannot be made from the generalized standard error of the odds that one category is either greater or less than another, since the generalized standard error may be too small for the category with the greater dispersion and too large for the one with the lesser dispersion. It would seem valid, however, to use not only the generalized standard error but also the various interactions to estimate the random sampling fluctuations about the general mean, since only the dispersion about the general mean is considered and not the differences between means of specific categories. Therefore, in this paper F values were used to determine trends and not differences between specific rates and dates.

In a study of this kind several factors may influence the final results. Some of these, such as extremely low temperatures and moisture, may destroy part or all of the experiment. In analyzing the data an effort was made to discover some of the causes affecting the yield of winter wheat planted at different rates and dates. In order to do this and to eliminate the effect of as many different factors as possible, yields which were definitely affected by winterkilling, date of emergence, and lack of moisture have not been included in some of the analyses.

For the foregoing reasons only data from years in which the crops survived the winter and in which moisture was sufficient to produce a crop have been used. The data for the years 1925 to 1928, inclusive, 1934, 1935, 1938, and 1939 were not used for the following reasons: In 1925 part of the plots were destroyed by army cutworms and no yields were obtained in 1926, 1927, and 1928 the 1-peck rate was not included in the test; in 1934 the crop on cornland was a failure; in 1935 poor stands were obtained and, regardless of date of planting, the wheat emerged at the same time in the spring; and in 1938 and 1939 different dates of planting were used.

EXPERIMENTAL RESULTS

The results were analyzed separately for fallow and cornland. Data for 13 years (table 1) were used in the statistical analysis of the plots grown on summer fallow and for 12 years for the plots grown on cornland. The average annual yields in bushels per acre on summer fallow and cornland are given in table 2. The data for the years shown in table 2 are representative of those for the entire 18 years of the experiment.

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TABLE 1.—*Average yield per acre of Kanred winter wheat sown on summer fallow at various rates on different dates, for 13 of the years between 1920 and 1937*

Seeding rate per acre (pecks)	Yields per acre for wheat sown—					
	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 15	Average
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*	*Bushels*
1	10.2	14.5	15.3	10.6	9.9	12.1
2	12.0	16.2	15.5	11.5	11.1	13.3
3	13.0	16.1	15.4	12.2	11.3	13.6
4	13.8	16.1	14.9	12.4	11.8	13.8
5	14.5	16.4	14.5	12.4	12.3	14.0
Average	12.7	15.9	15.1	11.8	11.3	13.4

TABLE 2.—*Yields per acre of Kanred wheat in rate- and date-of-seeding experiments on fallow and on cornland for certain years between 1920 and 1939*

FALLOW

Seeding dates	Seeding rate per acre	Yields per acre for wheat sown in—																		Average yield per acre for all years	Average number of years used in statistical analysis[1]
		1920	1921	1922	1923	1924	1926	1927	1928	1929	1930	1931	1932	1933	1934	1936	1937	1938	1939		
	Pecks	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Years*
Aug. 15–18	1	16.6	22.7	10.0	2.6	.0				13.3	12.2	4.2	14.5	4.4	.4	12.2	20.4	1.2	.9	[2] 9.04	10.27
	2	27.9	25.1	13.9	5.5	.0	2.9	19.0	24.1	14.8	18.4	2.7	16.3	4.4	.0	13.5	14.2	2.2	.4	[3] 11.41	12.05
	3	36.1	24.8	16.5	8.0	.0	.4	17.3	22.1	13.7	18.6	1.4	16.7	4.8	.0	14.5	14.0	4.8	1.2	[3] 11.93	13.00
	4	39.2	27.1	19.6	8.9	.0	.2	17.3	16.8	14.3	22.3	1.0	16.5	3.3	.0	14.0	12.8	6.6	1.6	[3] 12.31	13.77
	5	42.2	26.6	23.5	10.2	.0	.4	15.9	16.5	15.1	22.5	1.0	19.3	2.9	.0	14.7	10.5	7.1	1.4	[3] 12.97	14.50
	6						.4	10.8	18.4												
Average		32.4	25.3	16.7	7.0	.0	.9	16.1	19.6	14.2	18.8	2.0	16.6	3.9	.0	13.7	14.4	4.2	1.1	11.7	12.7
Sept. 1–4	1	39.5	10.1	19.1	9.6	3.5				14.4	25.2	3.1	20.4	1.9	.9	18.3	23.2	7.3	4.4	[2] 13.39	14.55
	2	43.9	13.7	19.8	12.2	6.1	.2	21.1	40.8	14.7	24.1	3.4	23.3	1.4	1.5	19.5	26.5	9.5	3.4	[3] 15.84	16.16
	3	40.3	15.2	21.6	12.0	6.6	.2	18.4	41.5	14.3	24.2	3.3	23.8	.9	1.1	19.3	27.0	11.6	2.8	[3] 15.78	16.12
	4	41.8	17.4	21.4	14.5	8.2	1.0	12.3	43.4	14.4	23.2	3.5	21.5	.9	.8	18.1	23.9	12.4	2.3	[3] 15.61	16.12
	5	43.8	15.7	23.3	15.4	9.6	1.0	10.5	42.7	13.4	19.8	5.6	22.9	.9	.7	17.7	24.3	13.7	1.8	[3] 15.71	16.39
	6						.8	9.6	42.2												
Average		41.9	14.4	21.1	12.7	6.8	.6	14.4	42.1	14.2	23.3	3.8	22.4	1.2	1.0	18.6	25.0	10.9	2.9	15.3	15.9
Sept. 15–18	1	26.3	13.7	18.3	13.3	10.7				13.7	21.1	7.2	22.8	2.2	5.3	17.9	26.2	20.2	6.9	[3] 15.05	15.28
	2	28.8	15.6	17.0	14.4	9.3	.8	17.1	46.3	13.7	19.8	7.0	25.2	2.2	4.3	19.4	24.9	21.5	10.8	[3] 16.56	15.51
	3	27.9	16.5	20.4	14.1	9.8	1.3	13.8	51.0	13.2	18.6	6.9	23.8	2.3	2.8	19.0	25.7	20.5	10.3	[3] 16.55	15.46
	4	28.5	15.2	19.7	12.9	8.8	.8	12.5	53.5	12.3	21.1	7.6	18.7	2.6	1.9	19.8	25.1	17.5	7.6	[3] 15.89	14.94
	5	23.4	15.6	18.7	13.9	9.0	.8	11.3	41.5	12.6	21.1	8.2	16.4	2.8	2.6	19.4	25.3	23.4	6.6	[3] 15.14	14.54
	6						.8	10.8	48.7												
Average		27.0	15.3	18.9	13.7	9.5	.9	13.1	48.2	13.1	20.3	7.4	21.3	2.4	3.3	19.1	25.4	20.6	8.4	15.8	15.1

Footnotes at end of table.

TABLE 2.—*Yields per acre of Kanred wheat in rate- and date-of-seeding experiments on fallow and on cornland for certain years between 1920 and 1939*—Continued

FALLOW

Seeding dates	Seeding rate per acre	Yields per acre for wheat sown in—																		Average yield per acre for all years	Average number of years used in statistical analysis
		1920	1921	1922	1923	1924	1926	1927	1928	1929	1930	1931	1932	1933	1934	1936	1937	1938	1939		
	Pecks	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Years*
Oct. 1–4	1	3.9	9.7	12.6	11.0	7.6	.....	.....	.....	11.7	17.5	8.3	15.3	4.5	5.8	10.1	19.5	16.2	3.0	[2] 10.45	10.58
	2	4.2	11.9	14.7	12.1	6.8	1.3	19.6	26.5	11.6	20.0	6.8	17.0	4.7	5.7	10.7	23.3	17.8	2.0	[3] 12.04	11.50
	3	6.7	15.5	16.7	12.8	7.6	1.3	20.0	40.6	11.1	20.4	7.9	18.3	4.4	6.0	10.7	21.2	16.1	5.2	[3] 13.47	12.25
	4	8.3	16.5	16.6	12.8	6.3	1.7	16.7	37.5	11.6	20.4	7.5	18.6	3.8	5.7	12.0	20.9	15.3	.....	[4] 13.66	12.38
	5	6.1	17.1	18.0	13.8	7.0	1.7	16.9	46.3	13.2	22.3	7.5	18.1	4.5	4.2	11.1	19.0	16.5	.....	14.31	12.45
	6	.....	.....	.....	.....	.....	.6	18.3	46.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	5.9	14.2	15.8	12.5	7.0	1.3	18.3	39.4	11.8	20.1	7.6	17.4	4.4	5.4	10.9	20.8	16.4	3.4	12.8	11.8
Oct. 15–18	1	5.4	14.0	14.1	11.9	5.1	.....	.....	.....	11.8	13.6	8.1	14.1	3.7	5.4	6.4	14.0	.....	.....	[2] 9.82	9.82
	2	6.8	16.1	16.3	11.9	3.9	.8	23.3	30.8	13.4	16.1	9.5	15.6	4.1	5.5	7.3	16.3	.....	.....	[5] 12.36	10.98
	3	7.8	17.3	17.2	13.7	5.3	1.0	19.6	31.6	12.0	15.3	7.9	15.4	3.9	5.7	7.7	16.1	.....	.....	[5] 12.34	11.18
	4	12.5	18.5	18.6	15.0	3.8	1.0	21.1	37.6	11.6	15.6	7.3	14.0	3.4	4.6	7.7	19.5	.....	.....	[5] 13.24	11.70
	5	12.8	17.4	19.4	14.3	4.4	1.3	22.7	32.3	11.8	16.5	7.5	14.8	4.5	5.0	9.0	20.9	.....	.....	[5] 13.41	12.18
	6	.....	.....	.....	.....	.....	.2	19.6	32.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	9.1	16.7	17.2	13.3	4.5	.9	21.3	32.9	12.1	17.1	8.0	14.8	3.9	5.2	7.6	17.3	.....	.....	12.2	11.3

CORNLAND

Aug. 15–18	1	7.6	10.1	5.0	6.8	.0	.....	.....	.....	7.1	16.6	10.6	7.6	.0	.....	5.8	11.0	3.5	.4	[6] 6.58	7.35
	2	10.6	10.9	5.8	10.1	.0	.8	30.0	17.3	7.8	26.3	10.3	8.2	.0	.....	6.3	12.9	5.6	.4	[4] 9.61	9.10
	3	16.4	13.7	9.3	11.8	.0	.8	21.3	16.9	8.9	26.7	9.2	9.0	.0	.....	6.6	13.6	5.4	.4	[4] 10.00	10.43
	4	19.4	13.8	13.0	11.8	.0	.8	17.1	11.7	8.9	27.5	9.0	10.0	.0	.....	5.9	12.6	6.0	.6	[4] 9.89	10.99
	5	23.2	14.1	15.2	13.3	.0	1.0	16.0	11.5	10.1	26.5	9.1	9.6	.0	.....	5.8	12.2	6.7	.4	[4] 9.92	11.59
	6	.....	.....	.....	.....	.....	.8	7.9	10.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	15.4	12.5	9.7	10.7	.0	.8	17.3	13.5	8.5	24.7	9.6	8.9	.0	.....	6.1	12.4	5.4	.4	9.2	9.9

	1	24.0	6.9	11.8	9.4	5.7															

Sept. 1–4	1	24.0	6.9	11.8	9.4	5.7				9.6	32.7	11.2	9.4	.0		5.6	8.8	5.0	.9	[6] 10.07	11.26
	2	30.4	8.1	13.1	10.1	4.7	.8	9.2	28.2	8.8	29.6	11.8	9.8	.0		6.5	6.9	7.1	.9	[4] 10.94	11.65
	3	38.6	9.1	13.6	9.3	4.7	.8	11.7	29.4	8.0	29.2	11.6	9.9	.0		5.7	6.1	6.5	.4	[4] 11.45	12.15
	4	40.0	10.0	12.5	12.7	4.7	.6	8.8	31.0	7.8	28.5	11.8	9.4	.0		3.7	6.0	7.1	.4	[4] 11.47	12.26
	5	42.2	8.7	14.1	12.5	3.0	.4	12.5	33.3	8.1	28.2	12.4	8.5	.0		4.0	5.3	7.8	.4	[4] 11.85	12.25
	6						.8	14.2	31.6												
Average		35.0	8.6	13.0	10.8	4.6	.6	11.3	30.7	8.4	29.6	11.8	9.4	.0		5.1	6.6	6.7	.6	11.2	11.9
Sept. 15–18	1	28.3	12.6	16.9	9.8	4.2				9.2	25.2	10.4	5.7	.0		5.9	8.3	10.2	.9	[6] 10.54	11.37
	2	32.9	9.2	16.4	10.7	3.1	1.3	10.0	19.9	7.5	26.0	10.0	6.2	.0		6.0	7.6	11.9	1.0	[4] 10.57	11.30
	3	32.2	11.9	13.5	12.1	3.7	1.3	8.3	19.1	7.2	21.9	9.7	3.4	.0		6.2	5.4	16.4	.9	[4] 10.19	10.60
	4	29.7	13.1	13.0	13.0	3.3	1.0	10.0	24.5	7.3	23.8	9.2	4.3	.0		6.0	4.1	17.2	.6	[4] 10.59	10.57
	5	25.7	13.9	14.2	12.8	3.5	.8	8.8	24.8	6.7	24.3	7.7	3.0	.0		6.0	4.2	14.8	.8	[6] 10.12	10.17
	6						.6	6.3	23.8												
Average		29.8	12.2	14.8	11.7	3.5	1.0	8.7	22.4	7.5	24.2	9.4	4.5	.0		6.0	5.9	14.1	.8	[1] 10.4	10.8
Oct. 1–4	1	3.5	8.8	8.9	7.7	3.8				7.6	18.1	7.6	1.9	.4		4.4	8.3	4.3	.0	[6] 6.09	6.75
	2	2.8	10.2	10.2	9.2	2.3	.8	7.5	8.3	6.5	18.3	6.4	2.4	.4		4.8	7.4	5.2	.0	[4] 6.04	6.74
	3	3.4	11.1	10.0	9.1	3.4	.8	7.9	14.3	7.0	18.6	6.8	1.9	.7		6.0	5.3	6.4	.0	[4] 6.63	6.94
	4	4.0	13.0	9.6	9.0	2.4	.6	5.8	14.8	8.3	15.2	7.2	2.0	.6		6.0	4.4	7.2		[3] 6.88	6.81
	5	4.5	11.0	9.8	12.6	3.4	.8	12.9	23.4	8.7	21.1	7.3	2.3	.5		6.9	3.5	6.1		[5] 8.42	7.63
	6						.4	15.4	26.0												
Average		3.7	10.8	9.7	9.5	3.0	.7	9.9	17.4	7.6	18.2	7.0	2.1	.5		5.6	5.7	5.8	.0	6.4	7.0
Oct. 15–18	1	4.8	10.1	5.8	7.5	1.9				7.2	15.0	5.5	1.8	.5		2.2	8.5			[7] 5.9	5.90
	2	7.6	12.2	7.5	8.8	2.4	1.5	13.3	7.0	7.8	14.9	7.0	1.5	.7		2.7	7.6			[2] 6.8	6.72
	3	9.7	12.3	7.6	9.6	2.4	1.9	12.9	7.2	7.3	15.6	6.9	1.7	.7		2.8	6.0			[2] 7.0	6.89
	4	9.8	13.3	9.0	9.1	2.6	1.7	10.8	10.6	7.3	16.6	7.0	1.4	.7		3.4	4.8			[2] 7.2	7.07
	5	12.4	17.8	10.6	11.1	2.6	2.1	10.4	11.5	7.3	13.8	7.2	1.2	.6		3.8	4.2			[2] 7.8	7.72
	6						1.9	12.5	11.0												
Average		8.9	13.2	8.1	9.2	2.4	1.8	12.0	9.5	7.4	15.2	6.7	1.5	.6		3.0	6.2			7.0	6.9

[1] 13-year average for fallow and a 12-year average for corn land.
[2] 15-year average.
[3] 18-year average.
[4] 17-year average.
[5] 16-year average.
[6] 14-year average.
[7] 12-year average.

RATE AND DATE OF SEEDING KANRED WINTER WHEAT
NO FALLOW

The analysis of variance for the plots grown on summer fallow is given in table 3. The *F* values indicate that there are significant differences in yield between rates and between dates of seeding, and that rates within dates tend to hold the same relative positions. Rates within years also show a tendency to remain constant. However, dates within years vary, and the same date may not give the highest yield every year.

TABLE 3.—*Analysis of variance for rates and dates of seeding Kanred winter wheat on summer fallow for 13 of the years between 1920 and 1937*

Variation due to—	Degrees of freedom	Sum of squares	Variance	Values of *F*, using for the divisor[1]—			
				Error	Rates × dates	Interaction with year	Rates
Years	12	29,356.893	2,446.408	229.688**			
Blocks	13	2,118.824	162.986	15.302**			
Rates	4	296.038	74.010	6.949**	5.567**	5.234**	
Dates	4	2,139.418	534.854	50.216**	40.233**	2.079	7.227*
Rates×dates	16	212.701	13.294	1.248			
Rates×years	48	678.752	14.141	1.328			
Dates×years	48	12,351.632	257.326	24.160**			
Rates×dates×years	192	1,293.943	6.739				
Error	312	3,323.036	10.651				
Total	649	51,771.237					

[1] * indicates significance at 5-percent level; ** at 1-percent level.

Using rates×years or dates×years as error, there is still a significant difference for rates of planting, but because of the variability within different years the different dates are not significant.

When the year effect is eliminated and dates×rates is used as error, there is a significant difference for both rates and dates. This indicates that on the average certain rates and certain dates give better yields, and that other rates and dates give poorer yields.

Dividing rates by dates, an *F* value of 7.227 was obtained, which is greater than the 5-percent point and indicates that dates are more important than rates.

The results are presented graphically in figure 2. This figure and table 1 indicate that the 1-peck rate gives the lowest yield, and that any increase in yield when seeding is at a higher rate than 2 pecks is slight and not significant. The September 1 and 15 dates give the highest yields, with a marked drop before and after these dates. From these results it may be concluded that when winter wheat is planted at the proper time, between September 1 and 15, 2 pecks is the best rate of seeding.

RATE AND DATE OF SEEDING KANRED WINTER WHEAT ON CORNLAND

In the studies on cornland an additional year, 1924, has been left out since no stands were obtained. The average yields were somewhat lower for wheat following corn than for wheat following fallow.

The average yields are given in table 4, and the variance analysis is presented in table 5.

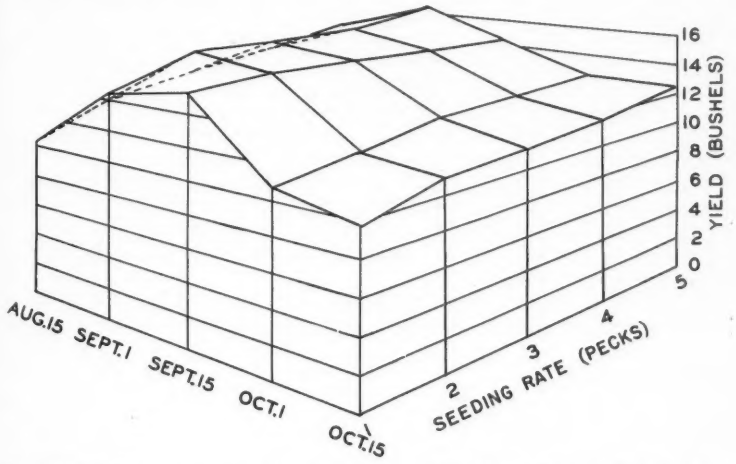

FIGURE 2.—Seeding rates, dates of sowing, and yields of Kanred winter wheat seeded on fallow.

TABLE 4.—*Average yields per acre of Kanred winter wheat sown on cornland at various rates on different dates for 12 years between 1920 and 1937*

Seeding rate per acre (pecks)	Yields per acre for wheat sown—					
	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 15	Average
1	7.3	11.2	11.4	6.7	5.9	8.5
2	9.1	11.6	11.3	6.7	6.7	9.1
3	10.4	12.1	10.6	6.9	6.9	9.4
4	11.0	12.3	10.7	6.8	7.1	9.5
5	11.6	12.3	10.2	7.6	7.7	9.9
Average	9.9	11.9	10.8	7.0	6.9	9.3

TABLE 5.—*Analysis of variance for rates and dates of seeding Kanred winter wheat on cornland for 12 of the years between 1920 and 1937*

Variation due to—	Degrees of freedom	Sum of squares	Variance	Values of F, using for the divisor[1]—			
				Error	Rates × dates	Interaction with year	Rates
Years	11	21,596.610	1,963.328	212.596**			
Blocks	12	5,214.775	434.565	47.056**			
Rates	4	125.158	31.290	3.388**	1.993	2.159	
Dates	4	2,493.107	623.277	67.491**	39.692**	3.539*	19.919**
Rates×dates	16	251.243	15.703	1.700			
Rates×years	44	637.552	14.490	1.569*			
Dates×years	44	7,748.353	176.099	19.069**			
Rates×dates×years	176	881.176	5.007				
Error	288	2,659.625	9.235				
Total	599	41,607.599					

[1] * indicates significance at 5-percent level; ** at 1-percent level.

The F value in table 5 shows that both rates and dates differ significantly. The F value for rates×dates indicates that the rates are fairly constant within dates. Both rates and dates vary within different years.

When the dates×year interaction is used as error, we find a significant F value for dates, indicating that there is a significant difference in yield due to dates of planting. However, when the rates× years interaction is used as error, the F value is not significant, indicating that yields due to rates of planting do not differ significantly. However, when the variability due to years is eliminated and the rates×dates interaction is used as error, there is a highly significant F value for dates and a low and not significant value for rates. As in the case of fallow, dates are again more important in planting winter wheat, as indicated by the F value of 19.919, which is greater than the 1-percent point.

The data in table 4 and the graphical representation of the data in figure 3 show a slight increase in yield on the average for seedings

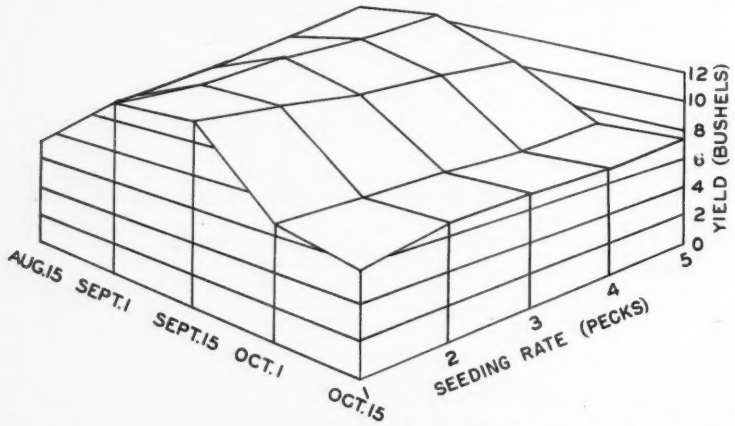

FIGURE 3.—Seeding rates, dates of sowing, and yields of Kanred winter wheat seeded on cornland.

above the 1-peck rate but quite a variation within dates. The data for date of planting show that planting on September 1 gave the highest average yield. This holds true throughout the range of rates, with the exception of the 1-peck rate, which is slightly higher on September 15. The early dates reverse their position for the 4- and 5-peck rates. However, basing results on average yield, it would seem safe to recommend that not to exceed 2 pecks of seed should be used and that the period from September 1 to 15 is the best time to plant winter wheat on cornland.

RELATION OF DRY-LAND FOOT ROT TO DATE OF PLANTING

Estimates of the amount and severity of infection with dry-land foot rot are given in table 6. Yields from adjacent plots seeded at the same rate in the rate-and-date studies on fallow are given in the

last column of the table. In all years infection and severity were greatest for the early seedings and descended progressively with the date of seeding. In studying the relationship between disease and yield, correlation coefficients were ascertained. In order to overcome the variability between different years, the yield for each date was calculated in percentage of the highest yield for that year. The highest yield for each year was considered as 100 percent, and the yields for the first three dates of planting were expressed as percentages of this value. By this means correlation coefficients of

TABLE 6.—*The relation of date of seeding Kanred winter wheat to development of dry-land foot rot, 1930–38, and yields from adjacent plots in the rate-and-date studies on fallow, seeded at approximately the same dates*

Dates		Dry-land foot rot readings on crowns		Yield per acre
Planted	Emerged	Plants diseased	Plant severity	
1930–31	*1930–31*	*Percent*	*Percent*	*Bushels*
Aug. 18	Aug. 24	100.0	100.0	1.4
Sept. 1	Sept. 8	94.0	80.0	3.3
Sept. 15	Sept. 23	92.0	10.0	6.9
Oct. 1	Oct. 10	86.0	2.0	7.9
Oct. 15	Nov. 10	46.0	Trace	7.9
1931–32	*1931–32*			
Aug. 15	Aug. 22	68.5	10.0	16.7
Sept. 1	Sept. 8	53.3	30.0	23.8
Sept. 15	Sept. 24	40.8	25.0	23.8
Oct. 1	Oct. 15	30.0	5.0	18.3
Oct. 15	Apr. 5	25.7	1.0	15.4
1932–33	*1932–33*			
Aug. 15	Aug. 22	90.0	25.0	4.8
Sept. 1	Sept. 8	94.0	20.0	.9
Sept. 15	Sept. 24	22.0	.5	2.3
Oct. 1	Oct. 11	.0	.0	4.4
Oct. 15	[1]	.0	.0	3.9
1933–34	*1933–34*			
Aug. 15	Aug. 28	97.6	75.0	.0
Sept. 1	Sept. 7	88.8	60.0	1.1
Sept. 15	Sept. 21	97.2	50.0	2.8
Oct. 1	Oct. 10	52.9	20.0	6.0
Oct. 15	Oct. 30	6.5	5.0	5.7
1934–35	*1934–35*			
Aug. 15	Aug. 21	48.8	12.1	.0
Sept. 4	Sept. 11	57.1	10.6	.0
1935–36	*1935–36*			
Aug. 15	Aug. 20	85.9	41.0	14.5
Sept. 1	Sept. 6	91.5	53.0	19.3
Sept. 15	Sept. 23	52.8	12.0	19.0
Oct. 1	Oct. 9	.0	.0	10.7
1936–37	*1936–37*			
Aug. 15	Aug. 20	95.7	50.5	14.0
Sept. 1	Sept. 9	84.6	32.5	27.0
Sept. 15	Sept. 23	51.4	17.9	25.7
Oct. 1	Oct. 15	23.2	5.0	21.2
Oct. 15	Nov. 1	10.5	5.0	16.1
1937–38	*1937–38*			
Aug. 16	Aug. 23	36.8		4.8
Sept. 1	Sept. 6	44.1		11.6
Sept. 15	Sept. 21	6.5		20.5
Oct. 1	Oct. 9	.0		16.1
1938–39	*1938–39*			
Aug. 15	Aug. 24	82.0	41.1	1.2
Sept. 1	Sept. 7	68.0	27.9	2.8
Sept. 15	Sept. 23	47.0	6.7	10.3
Oct. 1	Oct. 14	.0	.0	5.2

[1] Early winter.

−0.34 and −0.33 were found between yield in percentage of the highest yield and percentage of foot rot and severity, respectively. Neither correlation can be considered significant. The later dates of planting were not considered, since foot rot did not seem to be a factor in affecting the yield of October 1 and October 15 plantings. Table 6 shows that the percentage severity approaches zero for the October 1 and 15 plantings.

Since percentage infection and severity act together in the total damage done to the plant, a correlation between percentage infection times percentage severity and the percentage yield for the first three dates of planting based on the highest yield in each year was ascertained, and a correlation coefficient of −0.5588 with a p value of <0.01 was obtained (table 7), indicating a decrease in yield with an increase in disease. There was a decrease in yield after October 1, but this decrease was not caused by disease, as indicated by the data in table 6.

TABLE 7.—*Percentage of plants diseased × severity of infection, and yield of Kanred winter wheat in percentage of yield for the best date at the 3 peck rate of seeding for each year for 7 of the years between 1930 and 1938*

PERCENTAGE DISEASED × SEVERITY

Date of seeding	1930-31	1931-32	1932-33	1933-34	1935-36	1936-37	1938-39
Aug. 15	100	7	22	73	35	48	34
Sept. 1	75	16	19	53	48	27	19
Sept. 15	9	10	1	49	6	9	3

YIELD IN PERCENTAGE

Aug. 15	18	70	100	0	75	52	12
Sept. 1	42	100	19	18	100	100	27
Sept. 15	87	100	48	47	98	95	100

YIELD-TEMPERATURE RELATIONSHIPS

To find whether there is a critical temperature for planting winter wheat in order to obtain the highest yield, the relationship between the average "mean daily" temperature for the week before planting and the average yield in percentage of the highest yearly yield was ascertained for the August 15 and September 1 plantings.

In making these calculations the data from 1920 and 1930 were not used because of delayed emergence of wheat planted on the first two dates. The data from the years 1924, 1931, and 1933 were not included since the yields were all low because of lack of moisture. Table 8 gives the yields of Kanred wheat in percentage of the highest yearly yield calculated from the average of all rates within each date for each year and the average mean daily temperature for the week before planting. A correlation coefficient of −0.0898 was obtained. For the years studied this does not indicate any relationship between average mean daily temperature the week previous to planting on August 15 and September 1 and yield of Kanred winter wheat.

TABLE 8.—*Yield of Kanred wheat sown August 15 and September 1, expressed as the percentage of the highest yearly yield, and the average mean daily temperature the week preceding planting for 7 of the years between 1921 and 1937*

YIELD

Date	1920–21	1921–22	1922–23	1928–29	1931–32	1935–36	1936–37
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 15	100	79	51	100	74	72	57
Sept. 1	57	100	93	100	100	97	98

AVERAGE MEAN DAILY TEMPERATURE 1 WEEK PRECEDING PLANTING

	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
Aug. 8–15	65.9	71.4	74.6	73.9	70.9	77.6	78.5
Aug. 24–31	63.9	75.1	76.2	74.8	69.0	68.2	72.8

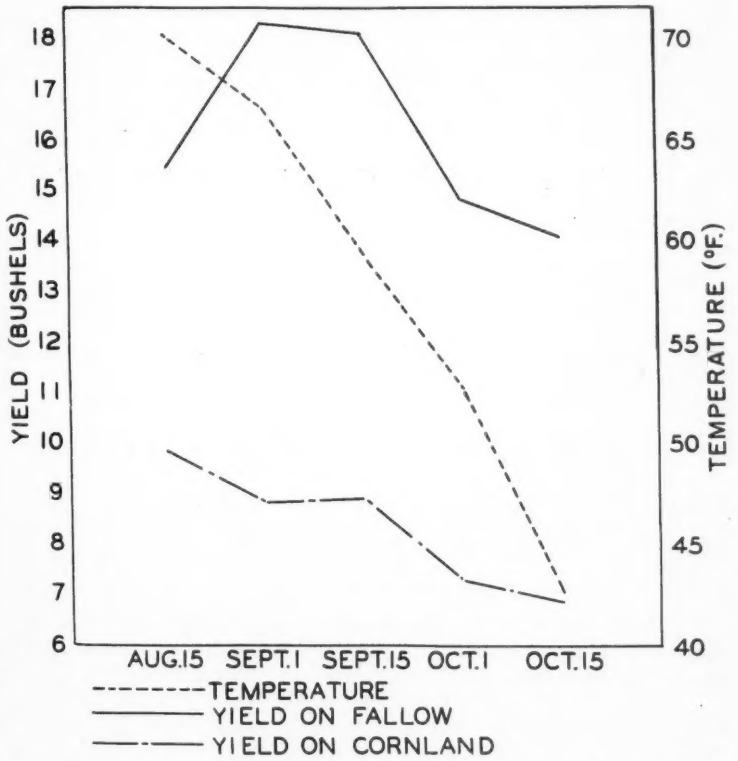

FIGURE 4.—Yield of Kanred winter wheat planted on fallow and on cornland at different dates and the average "mean daily" temperature for the first 3 weeks after planting.

Since the start of growth of wheat in the fall depends somewhat upon moisture and temperature after the wheat is planted, a further study was made in the years in which moisture was sufficient to

produce average yields. This was done to ascertain the influence of temperature for the first 3-week period after planting. The average mean daily temperature for this period was correlated with the yield in percentage of the highest yearly yield of winter wheat grown on fallow. From the data previously described, it is evident that there is an increase in yield up to September 1 and a sharp reduction in yield after September 15. In order to ascertain the effect of temperature on yield, the data from the different dates were divided into two periods: (1) August 15 to September 1, and (2) September 1 to October 15. Table 9 presents the yield data and the average mean daily temperatures for 7 of the years between 1921 and 1937. A significant negative correlation coefficient of -0.5498 was obtained for the yield and temperature relationship for the first two dates, and a significant positive correlation of $+0.5069$ was found for the four dates from September 1 to October 15. Figure 4 presents the data for the temperature and yield relationships on fallow and on cornland. The yield curve for the winter wheat planted on cornland drops off gradually throughout and does not show the negative effect of temperature for the early periods. This change is due to high yields for the August 15 planting in 1921 and 1937. When the entire period studied is considered, the data follow the same trend as that for wheat on fallow.

TABLE 9.—*Yield of Kanred winter wheat sown at different dates, expressed as the percentage of the highest yearly average yield for all rates of sowing, and the average mean daily temperatures for 3 weeks after each planting for 7 of the years between 1921 and 1937*

YIELD

Dates	1920–21	1921–22	1922–23	1928–29	1931–32	1935–36	1936–37
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 15	100	79	51	100	74	72	57
Sept. 1	57	100	93	100	100	97	98
Sept. 15	60	90	100	92	95	100	100
Oct. 1	56	75	91	83	78	57	82
Oct. 15	66	82	97	85	66	40	68

AVERAGE MEAN DAILY TEMPERATURE 3 WEEKS AFTER PLANTING

	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
Aug. 16–Sept. 7	63.6	70.4	74.9	70.1	70.7	68.0	73.0
Sept. 1–22	64.7	64.4	67.3	66.2	69.6	65.2	68.4
Sept. 16–Oct. 7	60.4	61.2	62.7	57.6	60.5	59.0	54.5
Oct. 1–22	54.2	56.7	51.7	51.6	54.2	52.1	48.7
Oct. 16–Nov. 7	40.7	49.9	45.0	39.0	49.6	37.9	37.0

In order to ascertain the best possible average mean daily temperature for the 3 weeks after planting, regressions were calculated for the two correlation coefficients. From this information lines were drawn (fig. 5), and the point of intersection of the two regression lines was found to be 65.8° F. These data indicate that under Colorado conditions the highest yield of winter wheat is most likely to be obtained when plantings are made on a date preceding a 3-week period in which the average daily mean temperature is 65.8° F. This temperature condition has occurred most often between September 1 and 15 during the years of the study. Other factors such as moisture and winter-killing may alter the results expected.

DISEASE-TEMPERATURE RELATIONSHIPS

Since a significant correlation coefficient was obtained for yield and temperature for a 3-week period after planting, and for yield in the early dates and disease infection × severity, it follows that there should be a relationship between temperature for the 3-week period after planting and disease infection × severity. Since the disease study included only data from 7 years between 1930 and 1938, the data for these 7 years of the study were used. Table 10 and figure 6

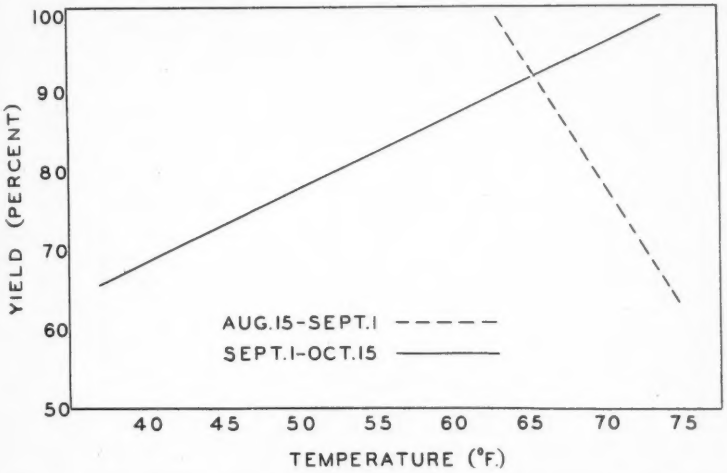

FIGURE 5.—Regression lines showing the most favorable average mean daily temperature for the 3 weeks after planting Kanred winter wheat. The point of intersection of the two lines is considered as the best possible estimate.

TABLE 10.—*Percentage of plants diseased × severity of infection, and the average mean daily temperature for 3 weeks after planting Kanred winter wheat for 7 of the years between 1931 and 1939*

PERCENTAGE DISEASED × SEVERITY

Dates	Years grown						
	1930–31	1931–32	1932–33	1933–34	1935–36	1936–37	1938–39
Aug. 15	100	7	22	73	35	48	34
Sept. 1	75	16	19	53	48	27	19
Sept. 15	9	10	1	49	6	9	3
Oct. 1	2	2	0	11	0	1	.....
Oct. 15	0	0	0	0	.....	1	.....

AVERAGE MEAN DAILY TEMPERATURE 3 WEEKS AFTER PLANTING

	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Aug. 16–Sept. 7	68.0	70.7	70.4	69.8	68.0	73.0	69.3
Sept. 1–Sept. 22	64.5	69.6	64.9	68.5	65.2	68.4	62.6
Sept. 16–Oct. 7	58.5	60.5	57.1	61.2	59.0	54.5	64.4
Oct. 1–Oct. 22	49.0	54.2	49.3	53.1	52.1	48.7	.....
Oct. 16–Nov. 7	41.6	49.6	41.7	46.4	37.9	37.0	.....

present the data. A positive correlation of +0.5919 with a p value of 0.01 was found for disease infection × severity and the average mean daily temperature for a 3-week period after planting for the following dates of planting: August 15, September 1, September 15, October 1, and October 15. This indicates a direct relationship between disease and temperature.

In order to find the type of regression curve which would best fit the data, both linear and quadratic regressions were calculated and the better fit ascertained by variance analysis (table 11).

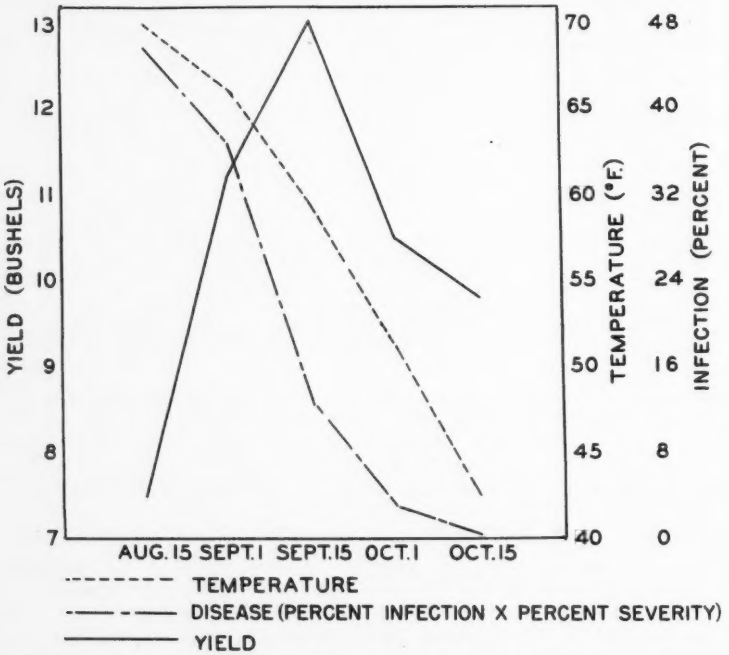

FIGURE 6.—Relationship of average mean daily temperature, percentage of infection × disease severity and yield of Kanred winter wheat planted at different dates in the fall.

DISCUSSION

There has long been a belief that temperature at the time of and after planting winter wheat effects the yield. The influence of temperature after planting on the prevalence of bunt in winter wheat when the seed has been inoculated with bunt spores is well known. Temperature after planting may influence the incidence and severity of dry-land foot rot in areas where it is an important factor in reducing yields, as indicated by the negative correlation coefficient of −0.5588, $p<0.01$, between infection in percent × severity in percent and yield in percent for the early planting dates, and the significant negative correlation between yield in percent and average daily mean tempera-

ture for a 3-week period after planting (table 9) for the early planting dates, August 15 and September 1.

The foregoing data, together with the positive correlation coefficient of $+0.5919$, $p<0.01$, between disease infection $\times$ severity and the average mean daily temperature for a 3-week period after planting, clearly indicate that temperature affects yield and that part of the reduction in yield in the early plantings is due to the effect of temperature on the incidence of dry-land foot rot. After September 15 the effect of foot-rot infection is reduced, and low temperature evidently is the main factor in reducing yields. This is indicated by the correlation coefficient of $+0.5069$, $p<0.01$, for the average mean daily temperature for the 3-week period after planting for the four dates of September 1, September 15, October 1, and October 15, and yield.

TABLE 11.—*Variance analysis comparing linear and quadratic regression of the percentage of disease $\times$ severity and temperature for the 3-week period after planting*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	[1]*F*
Quadratic—linear regression	1	11,008.45	11,008.45	224.8**
Deviation from quadratic regression	24	1,174.71	48.95	
Deviation from linear regression	25	12,183.16		

[1] **Greater than the 1-percent point. The analysis indicates that quadratic regression fits the data better than linear regression.

Moisture or lack of moisture, as previously shown, may be an important factor in influencing the growth and final yield of the winter wheat crop planted at different dates. In the early-planted crops where a heavy growth occurs in the fall, followed by a dry winter and spring, the plants may exhaust the soil moisture, and loss of stand and poor yields may result. With later dates of planting, this effect is reduced since time for growth in the fall is reduced. However, the fact that disease severity was greatest in the early plantings and that it was correlated negatively with yield indicates that disease was also an important factor in reducing yields.

The later-sown plots, while using less moisture, were retarded in growth, and temperature evidently had an important effect on this retardation, as indicated by the significant positive correlation between yield and temparature for the 3 weeks after planting. Wheat is known to go into a dormant period when the temperature drops below a certain point. The amount of growth made by the plant before this period would determine its ability to develop after growth started in the spring. The better-developed plants would have an advantage in the spring over those less well developed, and under favorable conditions would grow into larger and healthier plants.

SUMMARY

The data from two studies presented in the foregoing paper indicate:

That planting winter wheat at different dates results in significant differences in yields on summer-fallow in eastern Colorado.

Winter wheat should be planted between September 1 and 15 to obtain the best yields on either summer-fallow or cornland.

One peck of seed per acre is not sufficient for all conditions but 2 pecks are sufficient for both fallow and cornland. Three pecks or more do not increase yields enough to justify the use of the additional seed.

From studies made to discover the cause of the reduction in yield arising from the different dates of planting, the following conclusions are drawn:

The effect of high temperature on the incidence of dry-land foot rot and the resulting effect of the disease on the plant is one of the main factors in reducing yields in the earlier plantings (August 15).

Low temperature is the main factor in reducing yields in plantings made after September 15.

Other factors such as moisture and winter-killing may act independently of rates or dates of planting in reducing yields.

U. S. GOVERNMENT PRINTING OFFICE: 1942